

**RNA INTERFERENCE MEDIATED INHIBITION OF VASCULAR
EDOTHELIAL GROWTH FACTOR AND VASCULAR EDOTHELIAL
GROWTH FACTOR RECEPTOR GENE EXPRESSION USING SHORT
INTERFERING NUCLEIC ACID (siNA)**

5 This application is a continuation-in-part of McSwiggen, filed on September 16, 2003, USSN to be assigned, which is a continuation-in-part of McSwiggen, PCT/US03/05022, filed February 20, 2003, which claims the benefit of Beigelman USSN 60/358,580 filed February 20, 2002, of Beigelman USSN 60/363,124 filed March 11, 2002, of Beigelman USSN 60/386,782 filed June 6, 2002, of McSwiggen, USSN 10 60/393,796 filed July 3, 2002, of McSwiggen, USSN 60/399,348 filed July 29, 2002, of Beigelman USSN 60/406,784 filed August 29, 2002, of Beigelman USSN 60/408,378 filed September 5, 2002, of Beigelman USSN 60/409,293 filed September 9, 2002, and of Beigelman USSN 60/440,129 filed January 15, 2003, and which is a continuation-in-part of Pavco, USSN 10/306,747, filed November 27, 2002, which claims the benefit of 15 Pavco USSN 60/334461, filed November 30, 2001, a continuation-in-part of Pavco, USSN 10/287,949 filed November 4, 2002, and a continuation-in-part of Pavco, PCT/US02/17674 filed May 29, 2002. The instant application claims priority to all of the listed applications, which are hereby incorporated by reference herein in their entireties, including the drawings.

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Field Of The Invention

25 The present invention concerns compounds, compositions, and methods for the study, diagnosis, and treatment of conditions and diseases that respond to the modulation of vascular endothelial growth factor (VEGF) and/or vascular endothelial growth factor receptor (e.g., VEGFr1, VEGFr2 and/or VEGFr3) gene expression and/or activity. The present invention also concerns compounds, compositions, and methods relating to 30 conditions and diseases that respond to the modulation of expression and/or activity of genes involved in VEGF and VEGF receptor pathways. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against VEGF and VEGF receptor gene expression.

Background Of The Invention

The following is a discussion of relevant art pertaining to RNAi. The discussion is provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

5 RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806; Hamilton *et al.*, 1999, *Science*, 286, 950-951). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of
10 post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random
15 integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-
20 oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Hamilton *et al.*, *supra*; Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Hamilton *et al.*, *supra*; Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in
25 translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having
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sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, 5 were the first to observe RNAi in *C. elegans*. Bahramian and Zarbl, 1999, *Molecular and Cellular Biology*, 19, 274-283 and Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mammalian systems. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494, describe RNAi induced by introduction of duplexes of 10 synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when 15 containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these 20 studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end of the guide sequence (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA 25 (Nykanen *et al.*, 2001, *Cell*, 107, 309).

Studies have shown that replacing the 3'-terminal nucleotide overhanging segments of a 21-mer siRNA duplex having two nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported 30 to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). In addition, Elbashir *et al.*, *supra*, also report that substitution of siRNA with 2'-O-methyl nucleotides completely

abolishes RNAi activity. Li *et al.*, International PCT Publication No. WO 00/44914, and Beach *et al.*, International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom, however, neither 5 application postulates to what extent such modifications would be tolerated in siRNA molecules, nor provides any further guidance or examples of such modified siRNA. Kreutzer *et al.*, Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of 10 double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer *et al.* similarly fails to provide examples or guidance as to what 15 extent these modifications would be tolerated in siRNA molecules.

Parrish *et al.*, 2000, *Molecular Cell*, 6, 1977-1087, tested certain chemical 20 modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these 25 siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that RNAs with two phosphorothioate modified bases also had substantial decreases in effectiveness as RNAi. Further, Parrish *et al.* reported that phosphorothioate modification of more than two residues greatly destabilized the 30 RNAs *in vitro* such that interference activities could not be assayed. *Id.* at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and found that substituting deoxynucleotides for ribonucleotides produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. *Id.* In addition, the authors tested certain base modifications, including substituting, in sense and antisense 35 strands of the siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine. Whereas 4-thiouracil and 5-bromouracil substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand 40 resulted in a substantial decrease in RNAi activity as well.

The use of longer dsRNA has been described. For example, Beach *et al.*, International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe a *Drosophila in vitro* RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger of activating interferon response. Li *et al.*, International PCT Publication No. WO 00/44914, describe the use of specific dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe particular methods for introducing certain dsRNA molecules into cells for use in inhibiting gene expression. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT Publication No. 99/53050, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe specific DNA constructs for use in facilitating gene silencing in targeted organisms.

Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, *Molecular Cell*, 6, 1977-1087, describe specific chemically-modified siRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic characteristics of an organism using certain dsRNAs. Cogoni *et al.*, International PCT Publication No. WO 01/53475, describe certain methods for isolating a *Neurospora*

silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*, International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain 5 dsRNAs. Deak *et al.*, International PCT Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi in *Drosophila*. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe certain synthetic siRNA 10 constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide sequences using certain dsRNAs. Echeverri *et al.*, International PCT Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer *et al.*, 15 International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using RNAi. Graham *et al.*, International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, US 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long dsRNA (greater 20 than 25 nucleotide) constructs that mediate RNAi. Harborth *et al.*, 2003, Antisense & Nucleic Acid Drug Development, 13, 83-105, describe certain chemically and structurally modified siRNA molecules. Chiu and Rana, 2003, RNA, 9, 1034-1048, describe certain chemically and structurally modified siRNA molecules.

SUMMARY OF THE INVENTION

25 This invention relates to compounds, compositions, and methods useful for modulating the expression of genes, such as those genes associated with angiogenesis and proliferation, using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the expression and activity of vascular endothelial growth factor (VEGF) and/or vascular 30 endothelial growth factor receptor (e.g., VEGFr1, VEGFr2, VEGFr3) genes, or genes involved in VEGF and/or VEGFr pathways of gene expression and/or VEGF activity by RNA interference (RNAi) using small nucleic acid molecules. In particular, the instant

invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of VEGF and/or VEGFr genes. A siNA of the invention can be unmodified or chemically-modified. A siNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating VEGF and/or VEGFr gene expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA molecules through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake. Further, contrary to earlier published studies, siNA having multiple chemical modifications retains its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, target validation, genomic discovery, 15 genetic engineering, and pharmacogenomic applications.

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of gene(s) encoding proteins, such as vascular endothelial growth factor (VEGF) and/or vascular endothelial growth factor receptors (e.g., VEGFr1, VEGFr2, VEGFr3), associated with the maintenance and/or development of cancer and other proliferative diseases, such as genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in **Table I**, referred to herein generally as VEGF and/or VEGFr. The description below of the various aspects and embodiments of the invention is provided with reference to the exemplary VEGF and VEGFr (e.g., VEGFr1, VEGFr2, 20 VEGFr3) genes referred to herein as VEGF and VEGFr respectively. However, the various aspects and embodiments are also directed to other VEGF and/or VEGFr genes, such as mutant VEGF and/or VEGFr genes, splice variants of VEGF and/or VEGFr genes, other VEGF and/or VEGFr ligands and receptors. The various aspects and embodiments are also directed to other genes that are involved in VEGF and/or VEGFr 25 mediated pathways of signal transduction or gene expression that are involved in the progression, development, and/or maintenance of disease (e.g., cancer). These additional genes can be analyzed for target sites using the methods described for VEGF and/or VEGFr genes herein. Thus, the modulation of other genes and the effects of such

modulation of the other genes can be performed, determined, and measured as described herein.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a vascular endothelial growth factor (e.g., VEGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D) gene, wherein said siNA molecule comprises about 19 to about 21 base pairs.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a vascular endothelial growth factor receptor (e.g., VEGFr1, VEGFr2, and/or VEGFr3) gene, wherein said siNA molecule comprises about 19 to about 21 base pairs.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a VEGF gene, for example, wherein the VEGF gene comprises VEGF encoding sequence.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a VEGFr gene, for example, wherein the VEGFr gene comprises VEGFr encoding sequence.

In one embodiment, the invention features a siNA molecule having RNAi activity against VEGF and/or VEGFr RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having VEGF and/or VEGFr or other VEGF and/or VEGFr encoding sequence, such as those sequences having GenBank Accession Nos. shown in **Table I**. In another embodiment, the invention features a siNA molecule having RNAi activity against VEGF and/or VEGFr RNA, wherein the siNA molecule comprises a sequence complementary to an RNA having other VEGF and/or VEGFr encoding sequence, for example mutant VEGF and/or VEGFr genes, splice variants of VEGF and/or VEGFr genes, variants of VEGF and/or VEGFr genes with conservative substitutions, and homologous VEGF and/or VEGFr ligands and receptors. Chemical modifications as shown in **Tables III and IV** or otherwise described herein can be applied to any siNA construct of the invention.

In one embodiment, the invention features a siNA molecule having RNAi activity against VEGF and/or VEGFr RNA, wherein the siNA molecule comprises a sequence

complementary to any RNA having VEGF and/or VEGFr encoding sequence, such as those sequences having VEGF and/or VEGFr GenBank Accession Nos. shown in **Table I**. In another embodiment, the invention features a siNA molecule having RNAi activity against VEGF and/or VEGFr RNA, wherein the siNA molecule comprises a sequence 5 complementary to an RNA having other VEGF and/or VEGFr encoding sequence, for example, mutant VEGF and/or VEGFr genes, splice variants of VEGF and/or VEGFr genes, VEGF and/or VEGFr variants with conservative substitutions, and homologous VEGF and/or VEGFr ligands and receptors. Chemical modifications as shown in **Tables III and IV** or otherwise described herein can be applied to any siNA construct of the 10 invention.

In another embodiment, the invention features a siNA molecule having RNAi activity against a VEGF and/or VEGFr gene, wherein the siNA molecule comprises nucleotide sequence complementary to nucleotide sequence of a VEGF and/or VEGFr gene, such as those VEGF and/or VEGFr sequences having GenBank Accession Nos. 15 shown in **Table I** or other VEGF and/or VEGFr encoding sequence, such as mutant VEGF and/or VEGFr genes, splice variants of VEGF and/or VEGFr genes, variants with conservative substitutions, and homologous VEGF and/or VEGFr ligands and receptors. In another embodiment, a siNA molecule of the invention includes nucleotide sequence that can interact with nucleotide sequence of a VEGF and/or VEGFr gene and thereby 20 mediate silencing of VEGF and/or VEGFr gene expression, for example, wherein the siNA mediates regulation of VEGF and/or VEGFr gene expression by cellular processes that modulate the chromatin structure of the VEGF and/or VEGFr gene and prevent transcription of the VEGF and/or VEGFr gene.

In one embodiment, siNA molecules of the invention are used to down regulate or 25 inhibit the expression of soluble VEGF receptors (e.g. sVEGFr1 or sVEGFr2). Analysis of soluble VEGF receptor levels can be used to identify subjects with certain cancer types. These cancers can be amenable to treatment, for example, treatment with siNA molecules of the invention and any other chemotherapeutic composition. As such, analysis of soluble VEGF receptor levels can be used to determine treatment type and the 30 course of therapy in treating a subject. Monitoring of soluble VEGF receptor levels can be used to predict treatment outcome and to determine the efficacy of compounds and compositions that modulate the level and/or activity of VEGF receptors (see for example

Pavco USSN 10/438,493, incorporated by reference herein in its entirety including the drawings).

In another embodiment, the invention features a siNA molecule comprising nucleotide sequence, for example, nucleotide sequence in the antisense region of the 5 siNA molecule that is complementary to a nucleotide sequence or portion of sequence of a VEGF and/or VEGFr gene. In another embodiment, the invention features a siNA molecule comprising a region, for example, the antisense region of the siNA construct, complementary to a sequence comprising a VEGF and/or VEGFr gene sequence or a portion thereof.

10 In one embodiment, the antisense region of VEGFr1 siNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOs. 1-427, 1997-2000, 2009-2012, or 2244-2255. In one embodiment, the antisense region can also comprise sequence having any of SEQ ID NOs. 428-854, 2024-2027, 2032-2035, 2040-2043, 2188-2190, 2197-2200, 2203, 2217, 2278-2280, 2292-2298, 2313-2318, 2326-2332, 15 2347-2364, 2448, 2450, 2452, or 2455. In another embodiment, the sense region of VEGFr1 constructs can comprise sequence having any of SEQ ID NOs. 1-427, 1997-2000, 2009-2012, 2020-2023, 2028-2031, 2036-2039, 2185-2187, 2201-2202, 2218, 2220, 2222, 2224, 2244-2255, 2275-2277, 2281-2291, 2299-2305, 2319-2325, 2333-2339, 2347-2364, 2447, 2449, 2451, 2453, or 2454. The sense region can comprise a 20 sequence of SEQ ID NO. 2438 and the antisense region can comprise a sequence of SEQ ID NO. 2439. The sense region can comprise a sequence of SEQ ID NO. 2440 and the antisense region can comprise a sequence of SEQ ID NO. 2441. The sense region can comprise a sequence of SEQ ID NO. 2442 and the antisense region can comprise a sequence of SEQ ID NO. 2443. The sense region can comprise a sequence of SEQ ID 25 NO. 2444 and the antisense region can comprise a sequence of SEQ ID NO. 2441. The sense region can comprise a sequence of SEQ ID NO. 2445 and the antisense region can comprise a sequence of SEQ ID NO. 2441. The sense region can comprise a sequence of SEQ ID NO. 2444 and the antisense region can comprise a sequence of SEQ ID NO. 2446.

30 In one embodiment, the antisense region of VEGFr2 siNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOs. 855-1178, 2001-

2004, or 2017-2019 or 2256-2271. In one embodiment, the antisense region can also comprise sequence having any of SEQ ID NOS. 1179-1502, 2048-2051, 2056-2059, 2064-2067, 2208-2210, 2214-2216, 2226-2227, 2230-2231, 2377-2388, 2391-2392, 2401-2405, or 2420-2423. In another embodiment, the sense region of VEGFr2
5 constructs can comprise sequence having any of SEQ ID NOS. 855-1178, 2001-2004, 2017-2019, 2256-2271, 2044-2047, 2052-2055, 2060-2063, 2205-2207, 2211-2213, 2228-2229, 2365-2376, 2389-2390, 2393-2394, 2397-2400, 2406-2410, 2416-2419, or 2424-2427. The sense region can comprise a sequence of SEQ ID NO. 2438 and the antisense region can comprise a sequence of SEQ ID NO. 2439. The sense region can
10 comprise a sequence of SEQ ID NO. 2440 and the antisense region can comprise a sequence of SEQ ID NO. 2441. The sense region can comprise a sequence of SEQ ID NO. 2442 and the antisense region can comprise a sequence of SEQ ID NO. 2443. The sense region can comprise a sequence of SEQ ID NO. 2444 and the antisense region can comprise a sequence of SEQ ID NO. 2441. The sense region can comprise a sequence of SEQ ID NO. 2445 and the antisense region can comprise a sequence of SEQ ID NO.
15 2441. The sense region can comprise a sequence of SEQ ID NO. 2444 and the antisense region can comprise a sequence of SEQ ID NO. 2446.

In one embodiment, the antisense region of VEGFr3 siNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOS. 1503-1749, 2005-
20 2008, or 2272-2274. In one embodiment, the antisense region can also comprise sequence having any of SEQ ID NOS. 1750-1996, 2072-2075, 2080-2083, 2088-2091, or 2435-2437. In another embodiment, the sense region of VEGFr3 constructs can comprise sequence having any of SEQ ID NOS. 1503-1749, 2005-2008, 2068-2071, 2076-2079, or 2084-2087, 2272-2274, or 2432-2434. The sense region can comprise a
25 sequence of SEQ ID NO. 2438 and the antisense region can comprise a sequence of SEQ ID NO. 2439. The sense region can comprise a sequence of SEQ ID NO. 2440 and the antisense region can comprise a sequence of SEQ ID NO. 2441. The sense region can comprise a sequence of SEQ ID NO. 2442 and the antisense region can comprise a sequence of SEQ ID NO. 2443. The sense region can comprise a sequence of SEQ ID NO.
30 NO. 2444 and the antisense region can comprise a sequence of SEQ ID NO. 2441. The sense region can comprise a sequence of SEQ ID NO. 2445 and the antisense region can comprise a sequence of SEQ ID NO. 2441. The sense region can comprise a sequence of

SEQ ID NO. 2444 and the antisense region can comprise a sequence of SEQ ID NO. 2446.

In one embodiment, a siNA molecule of the invention comprises any of SEQ ID NOs. 1-2455. The sequences shown in SEQ ID NOs: 1-2455 are not limiting. A siNA molecule of the invention can comprise any contiguous VEGF and/or VEGFr sequence (e.g., about 19 to about 25, or about 19, 20, 21, 22, 23, 24 or 25 contiguous VEGF and/or VEGFr nucleotides).

In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank Accession Nos. shown in **Table I**. Chemical modifications in **Tables III and IV** and described herein can be applied to any siRNA construct of the invention.

In one embodiment of the invention a siNA molecule comprises an antisense strand having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein the antisense strand is complementary to a RNA sequence encoding a VEGF and/or VEGFr protein, and wherein said siNA further comprises a sense strand having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29) nucleotides, and wherein said sense strand and said antisense strand are distinct nucleotide sequences with at least about 19 complementary nucleotides.

In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29) nucleotides, wherein the antisense region is complementary to a RNA sequence encoding a VEGF and/or VEGFr protein, and wherein said siNA further comprises a sense region having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or more) nucleotides, wherein said sense region and said antisense region comprise a linear molecule with at least about 19 complementary nucleotides.

In one embodiment of the invention a siNA molecule comprises an antisense strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a VEGF and/or VEGFr protein. The siNA further

comprises a sense strand, wherein said sense strand comprises a nucleotide sequence of a VEGF and/or VEGFr gene or a portion thereof.

In another embodiment, a siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence 5 encoding a VEGF and/or VEGFr protein or a portion thereof. The siNA molecule further comprises a sense region, wherein said sense region comprises a nucleotide sequence of a VEGF and/or VEGFr gene or a portion thereof.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a VEGFr gene. Because VEGFr genes can 10 share some degree of sequence homology with each other, siNA molecules can be designed to target a class of VEGFr genes (and associated receptor or ligand genes) or alternately specific VEGFr genes by selecting sequences that are either shared amongst different VEGFr targets or alternatively that are unique for a specific VEGFr target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved 15 regions of VEGFr RNA sequence having homology between several VEGFr genes so as to target several VEGFr genes (e.g., VEGFr1, VEGFr2 and/or VEGFr3, different VEGFr isoforms, splice variants, mutant genes etc.) with one siNA molecule. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific VEGFr RNA sequence due to the high degree of specificity that the siNA 20 molecule requires to mediate RNAi activity.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a VEGF gene. Because VEGF genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of VEGF genes (and associated receptor or ligand genes) or 25 alternately specific VEGF genes by selecting sequences that are either shared amongst different VEGF targets or alternatively that are unique for a specific VEGF target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of VEGF RNA sequence having homology between several VEGF genes so as to target several VEGF genes (e.g., VEGF-A, VEGF-B, VEGF-C and/or VEGF-D, 30 different VEGF isoforms, splice variants, mutant genes etc.) with one siNA molecule. In another embodiment, the siNA molecule can be designed to target a sequence that is

unique to a specific VEGF RNA sequence due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double-stranded nucleic acid molecules. In another embodiment, the siNA molecules of the invention consist of duplexes containing about 19 base pairs between oligonucleotides comprising about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24 or 25) nucleotides. In yet another embodiment, siNA molecules of the invention comprise duplexes with overhanging ends of about 1 to about 3 (e.g., about 1, 2, or 3) nucleotides, for example, about 21-10 nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs.

In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for VEGF and/or VEGFr expressing nucleic acid molecules, such as RNA encoding a VEGF and/or VEGFr protein. Non-limiting 15 examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, are shown to 20 preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, contrary to the data published by Parrish *et al.*, *supra*, applicant demonstrates that multiple (greater than one) phosphorothioate substitutions are well-tolerated and confer substantial increases in serum stability for modified siNA constructs.

25 In one embodiment, a siNA molecule of the invention comprises modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA 30 molecule. As such, a siNA molecule of the invention can generally comprise about 5% to about 100% modified nucleotides (e.g., 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%,

45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides). The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number 5 of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and antisense strands.

One aspect of the invention features a double-stranded short interfering nucleic 10 acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene. In one embodiment, a double stranded siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long. In one embodiment, the double-stranded siNA molecule does not contain any 15 ribonucleotides. In another embodiment, the double-stranded siNA molecule comprises one or more ribonucleotides. In one embodiment, each strand of the double-stranded siNA molecule comprises about 19 to about 23 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein each strand comprises about 19 nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, one of the 20 strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the VEGF and/or VEGFr gene, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of the VEGF and/or 25 VEGFr gene or a portion thereof.

In another embodiment, the invention features a double-stranded short interfering 25 nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene comprising an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of the VEGF and/or VEGFr gene or a portion thereof, and a sense region, wherein the sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence of the VEGF 30 and/or VEGFr gene or a portion thereof. In one embodiment, the antisense region and the sense region each comprise about 19 to about 23 (e.g. about 19, 20, 21, 22, or 23)

nucleotides, wherein the antisense region comprises about 19 nucleotides that are complementary to nucleotides of the sense region.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the VEGF and/or VEGFr gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. The sense region can be connected to the antisense region via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the VEGF and/or VEGFr gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the siNA molecule has one or more modified pyrimidine and/or purine nucleotides. In one embodiment, the pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides or 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In one embodiment, the pyrimidine nucleotides in the antisense region are 2'-deoxy-2'-fluoro pyrimidine

nucleotides and the purine nucleotides present in the antisense region are 2'-O-methyl or 2'-deoxy purine nucleotides. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the sense strand (e.g. overhang region) are 2'-deoxy nucleotides.

5 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein the fragment 10 comprising the sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment. In another embodiment, the terminal cap moiety is an inverted deoxy abasic moiety or glyceryl moiety. In another embodiment, each of the two fragments of the siNA molecule comprise about 21 nucleotides.

15 In one embodiment, the invention features a siNA molecule comprising at least one modified nucleotide, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. The siNA can be, for example, of length between about 12 and about 36 nucleotides. In another embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In another embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine 20 nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In another embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In another embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In another embodiment, all adenosine nucleotides 25 present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In another embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In another embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA 30 that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides. In another embodiment, the siNA comprises a sequence that is

complementary to a nucleotide sequence in a separate RNA, such as a VEGF or VEGFr RNA.

In one embodiment, the invention features a method of increasing the stability of a siNA molecule against cleavage by ribonucleases comprising introducing at least one 5 modified nucleotide into the siNA molecule, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. In another embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In another embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-10 deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In another embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In another embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In another 15 embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In another embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In another embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected 20 locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA 25 encoded by the VEGF and/or VEGFr gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the purine nucleotides present in the antisense region comprise 2'-deoxy- purine nucleotides. In an alternative embodiment, the purine nucleotides present in the antisense region comprise 2'-O-methyl purine nucleotides. In either of the above 30 embodiments, the antisense region can comprise a phosphorothioate internucleotide linkage at the 3' end of the antisense region. Alternatively, in either of the above embodiments, the antisense region can comprise a glyceryl modification at the 3' end of

the antisense region. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the antisense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary 5 nucleotides of the other fragment of the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In one embodiment, each of the 10 two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In another embodiment, all 15 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the VEGF and/or VEGFr gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the 20 nucleotide sequence or a portion thereof of the RNA encoded by the VEGF and/or VEGFr gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally include a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a VEGF and/or VEGFr RNA sequence (e.g., wherein said target RNA sequence is encoded by a VEGF and/or VEGFr gene involved in the VEGF and/or VEGFr pathway), wherein the siNA molecule does not contain any ribonucleotides and wherein each strand of the double-stranded siNA molecule is about 21 nucleotides long. Examples of non-ribonucleotide containing siNA constructs are combinations of stabilization chemistries shown in Table IV in any 25 combination of Sense/Antisense chemistries, such as Stab 7/8, Stab 7/11, Stab 8/8, Stab 30 18/8, Stab 18/11, Stab 12/13, Stab 7/13, or Stab 18/13.

In one embodiment, the invention features a medicament comprising a siNA molecule of the invention.

In one embodiment, the invention features an active ingredient comprising a siNA molecule of the invention.

5 In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule to down-regulate expression of a VEGF and/or VEGFr gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long.

10 In one embodiment, a VEGFr gene contemplated by the invention is a VEGFr1, VEGFr2, or VEGFr3 gene.

15 In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

20 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, the VEGFr gene is VEGFr2. In one embodiment, the VEGFr gene is VEGFr1.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA that encodes a protein or portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, each strand of the siNA molecule comprises about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand. In another embodiment, the siNA molecule is assembled from two oligonucleotide fragments, wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and a second fragment comprises nucleotide sequence of the sense region of the siNA molecule. In yet another embodiment, the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker. In a further embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In still another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-deoxy purine nucleotides. In another embodiment, the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides

and one or more 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides. In a further embodiment the sense strand comprises a 3'-end and a 5'-end, wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety or inverted deoxy nucleotide moiety such as inverted thymidine) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In another embodiment, the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the antisense strand comprises a glyceryl modification at the 3' end. In another embodiment, the 5'-end of the antisense strand optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein each of the two strands of the siNA molecule comprises about 21 nucleotides. In one embodiment, about 21 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 19 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule, wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine, such as 2'-deoxy-thymidine. In another embodiment, each strand of the siNA molecule is base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 19 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the VEGF and/or VEGFr RNA or a portion thereof. In another embodiment, about 21 nucleotides of the antisense

strand are base-paired to the nucleotide sequence of the VEGF and/or VEGFr RNA or a portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, 5 wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the 10 double-stranded siNA molecule comprises a sugar modification, and wherein the 5'-end of the antisense strand optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand 15 which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the 20 nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the untranslated region or a portion thereof of the VEGF and/or VEGFr RNA.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, 25 wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and 30 wherein the nucleotide sequence of the antisense strand is complementary to a nucleotide

sequence of the VEGF and/or VEGFr RNA or a portion thereof that is present in the VEGF and/or VEGFr RNA.

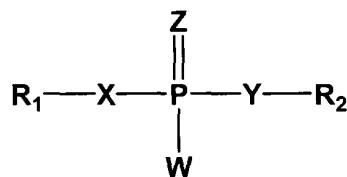
In one embodiment, the invention features a composition comprising a siNA molecule of the invention in a pharmaceutically acceptable carrier or diluent.

5 In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given
10 therapeutic effect since chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid molecule is reduced as compared to a native nucleic
15 acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than that of the native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siNA, chemically-modified siNA can also minimize the possibility of activating interferon activity in humans.

20 In any of the embodiments of siNA molecules described herein, the antisense region of a siNA molecule of the invention can comprise a phosphorothioate internucleotide linkage at the 3'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of said antisense
25 region. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs of a siNA molecule of the invention can comprise ribonucleotides or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides.
30 In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

One embodiment of the invention provides an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The 5 mammalian cell can be a human cell. The siNA molecule of the expression vector can comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or DNA sequence encoding VEGF and/or VEGFr and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having complementary sense and 10 antisense regions. The siNA molecule can comprise a single strand having complementary sense and antisense regions.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical 15 modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides comprising a backbone modified internucleotide linkage having Formula I:

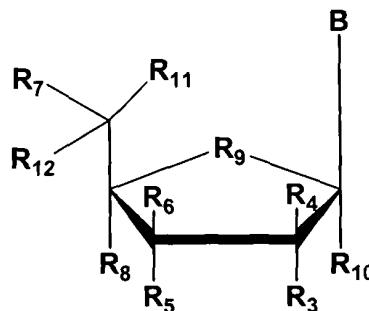


wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or 20 polynucleotide which can be naturally-occurring or chemically-modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or acetyl and wherein W, X, Y, and Z are optionally not all O. In another embodiment, a backbone modification of the invention comprises a phosphonoacetate and/or thiophosphonoacetate internucleotide linkage (see for example Sheehan et al., 2003, Nucleic Acids Research, 31, 4109-4118).

25 The chemically-modified internucleotide linkages having Formula I, for example, wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can

comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula II:

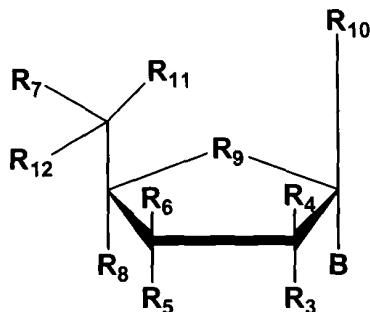


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF₃, OCF₃, OCN, O-alkyl, S-alkyl, 25 N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO₂, NO₂, N3,

NH₂, aminoalkyl, aminoacid, aminoacyl, ONH₂, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH₂, S=O, CHF, or CF₂, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

10 The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

25 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:

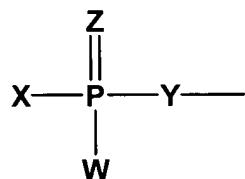


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF₃, OCF₃, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, 5
O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO₂, NO₂, N₃, NH₂, aminoalkyl, aminoacid, aminoacyl, ONH₂, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH₂, S=O, CHF, or CF₂, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-10 aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

15 The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense 20 strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., 25 about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

5 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, alkylhalo, or acetyl; and wherein W, X, Y and Z are not all O.

In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand, for example, a strand complementary to a target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (e.g., about 1, 2, or 20 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (e.g., about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

25 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical

modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another 5 embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules 10 of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense 15 strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 20 8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 25 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate 30 internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the

antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, 5 phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, or more) 2'-deoxy, 10 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 15 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA 20 strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

25 In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and 30 optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate

internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule having about 1 to about 5, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages in each strand of the siNA molecule.

In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the 2'-5' internucleotide linkage(s) can be present at various other positions within one or 5 both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

10 In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified, wherein each strand is about 18 to about 27 (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27) nucleotides in length, wherein the duplex has about 18 to about 23 (e.g., about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the chemical modification 15 comprises a structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and 20 wherein the duplex has about 19 base pairs. In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36 to about 70 (e.g., about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (e.g., about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of 25 Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 42 to about 50 (e.g., about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having 30 about 19 base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the invention is designed such that degradation of the loop

portion of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In another embodiment, a siNA molecule of the invention comprises a hairpin structure, wherein the siNA is about 25 to about 50 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 3 to about 23 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In another embodiment, a linear hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

In another embodiment, a siNA molecule of the invention comprises an asymmetric hairpin structure, wherein the siNA is about 25 to about 50 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 20 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms an asymmetric hairpin

structure having about 3 to about 18 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In another embodiment, an asymmetric hairpin siNA molecule of the invention 5 contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In another embodiment, an asymmetric hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

In another embodiment, a siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising 10 sense and antisense regions, wherein the antisense region is about 16 to about 25 (e.g., about 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length, wherein the sense region is about 3 to about 18 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) nucleotides in length, wherein the sense region and the antisense region have at 15 least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 18 to about 22 (e.g., about 18, 19, 20, 21, or 22) nucleotides in length and 20 wherein the sense region is about 3 to about 15 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) nucleotides in length, wherein the sense region the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. In another embodiment, the asymmetric double stranded siNA 25 molecule can also have a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV).

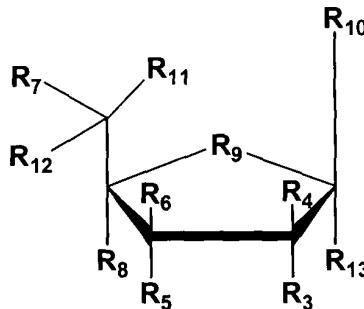
In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (e.g., about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (e.g., about 18, 30 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of

the invention comprises a circular oligonucleotide having about 42 to about 50 (e.g., about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 5 base pairs and 2 loops.

In another embodiment, a circular siNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable. For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule *in vivo* can generate a double-stranded siNA 10 molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

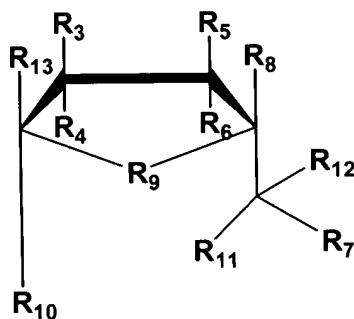
In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having Formula V:

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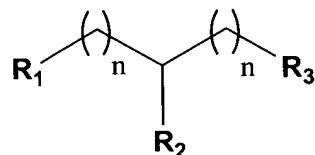
wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF₃, OCF₃, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-O-SH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO₂, 20 NO₂, N₃, NH₂, aminoalkyl, aminoacid, aminoacyl, ONH₂, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH₂, S=O, CHF, or CF₂.

In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a 25 compound having Formula VI:



wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and either R2, R3, R8 or R13 serve as points of attachment to the siNA molecule of the invention.

In another embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for example a compound having Formula VII:



wherein each n is independently an integer from 1 to 12, each R1, R2 and R3 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or a group having Formula I, and R1, R2 or R3 serves as points of attachment to the siNA molecule of the invention.

In another embodiment, the invention features a compound having Formula VII, wherein R1 and R2 are hydroxyl (OH) groups, n = 1, and R3 comprises O and is the point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for example modification 6 in **Figure 10**).

In another embodiment, a moiety having any of Formula V, VI or VII of the invention is at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, a moiety having Formula V, VI or VII can be present at the 10 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and sense strands of the siNA molecule. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

In another embodiment, a siNA molecule of the invention comprises an abasic 15 residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, a siNA molecule of the invention comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for 20 example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In another embodiment, a siNA molecule of the invention comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA 25 molecule.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-30 2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are

2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

5 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 10 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-15 deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 20 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

25 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 30 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (e.g., wherein

all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

5 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine 10 nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

15 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

25 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-deoxy purine

nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein

5 any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine

10 nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system

15 comprising a sense region, wherein one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g.,

20 wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and an antisense region, wherein one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-

25 2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). The sense region and/or the antisense region can have a terminal cap modification, such as any modification described herein or shown in **Figure**

30 **10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense and/or antisense sequence. The sense and/or antisense region can optionally further comprise a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about

1, 2, 3, or 4) 2'-deoxynucleotides. The overhang nucleotides can further comprise one or more (e.g., about 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in **Figures 4 and 5 and Tables III and IV** herein.

5 In any of these described embodiments, the purine nucleotides present in the sense region are alternatively 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides) and one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all

10 purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Also, in any of these embodiments, one or more purine nucleotides present in the sense region are alternatively purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or alternately a plurality of purine nucleotides are purine ribonucleotides) and any purine

15 nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Additionally, in any of these embodiments, one or more purine nucleotides present in the sense region and/or present in the antisense region are alternatively selected from the group consisting of 2'-

20 deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group

25 consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides).

In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As

such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-
5 limiting examples of nucleotides having a northern configuration include locked nucleic acid (LNA) nucleotides (e.g., 2'-O, 4'-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, and 2'-O-methyl nucleotides.

10 In one embodiment, the sense strand of a double stranded siNA molecule of the invention comprises a terminal cap moiety, (see for example **Figure 10**) such as an inverted deoxyabasic moiety, at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand.

In one embodiment, the invention features a chemically-modified short interfering
15 nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. Non-limiting examples of conjugates contemplated by the invention include conjugates and ligands described in Vargeese *et al.*, USSN 10/427,160, filed April 30,
20 2003, incorporated by reference herein in its entirety, including the drawings. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In another embodiment, the conjugate
25 molecule is attached at the 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention
30 comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a poly ethylene glycol, human

serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake. Examples of specific conjugate molecules contemplated by the instant invention that can be attached to chemically-modified siNA molecules are described in Vargeese *et al.*, U.S. Serial No. 10/201,394, incorporated by reference herein. The type of conjugates 5 used and the extent of conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine whether the siNA conjugate complex possesses 10 improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the 15 siNA to the antisense region of the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of ≥ 2 nucleotides in length, for example about 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid aptamer. By “aptamer” or “nucleic acid aptamer” as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic 20 acid molecule has sequence that comprises a sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of 25 the naturally occurring ligand with the protein. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, 30 *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628.)

In yet another embodiment, a non-nucleotide linker of the invention comprises abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid,

polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, 18:6353 and *Nucleic Acids Res.* 1987, 15:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, 113:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, 113:5109; Ma *et al.*, *Nucleic Acids Res.* 1993, 21:2585 and *Biochemistry* 1993, 32:1751; Durand *et al.*, *Nucleic Acids Res.* 1990, 18:6353; McCurdy *et al.*, *Nucleosides & Nucleotides* 1991, 10:287; Jschke *et al.*, *Tetrahedron Lett.* 1993, 34:301; Ono *et al.*, *Biochemistry* 1991, 30:9914; Arnold *et al.*, International Publication No. WO 89/02439; Usman *et al.*, International Publication No. 10 WO 95/06731; Dudycz *et al.*, International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, 113:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to 15 exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in 20 a *vitro* system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA comprise separate oligonucleotides not having any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotides. In another 25 example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA are linked or circularized by a nucleotide or non-nucleotide linker as described herein, wherein the oligonucleotide does not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly found that the presence of ribonucleotides (e.g., nucleotides 30 having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof

to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2',3'-cyclic phosphate). In another embodiment, the 10 single stranded siNA molecule of the invention comprises about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one or more chemically modified nucleotides or non-nucleotides described herein. For example, all the positions within the siNA molecule can include chemically-modified nucleotides 15 such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system comprising 20 a single stranded polynucleotide having complementarity to a target nucleic acid sequence, wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present 25 in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence. The siNA 30 optionally further comprises about 1 to about 4 or more (e.g., about 1, 2, 3, 4 or more) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, 4 or more) phosphorothioate,

phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group. In any of these embodiments, any purine nucleotides present in the antisense region are alternatively 2'-deoxy purine nucleotides (e.g., wherein all purine 5 nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA (i.e., purine nucleotides present in the sense and/or antisense region) can alternatively be locked nucleic acid (LNA) nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine 10 nucleotides are LNA nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA are alternatively 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-methoxyethyl purine nucleotides). In another embodiment, any modified nucleotides present in the single stranded siNA 15 molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present 20 in the single stranded siNA molecules of the invention are preferably resistant to nuclelease degradation while at the same time maintaining the capacity to mediate RNAi.

In one embodiment, the invention features a method for modulating the expression of a VEGF and/or VEGFr gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA 25 strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the cell.

In one embodiment, the invention features a method for modulating the expression of a VEGF and/or VEGFr gene within a cell comprising: (a) synthesizing a siNA 30 molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene and wherein the sense strand sequence of the siNA comprises a sequence identical or

substantially similar to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the cell.

In another embodiment, the invention features a method for modulating the
5 expression of more than one VEGF and/or VEGFr gene within a cell comprising: (a)
synthesizing siNA molecules of the invention, which can be chemically-modified,
wherein one of the siNA strands comprises a sequence complementary to RNA of the
VEGF and/or VEGFr genes; and (b) introducing the siNA molecules into a cell under
conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the
10 cell.

In another embodiment, the invention features a method for modulating the
expression of two or more VEGF and/or VEGFr genes within a cell comprising: (a)
synthesizing one or more siNA molecules of the invention, which can be chemically-
modified, wherein the siNA strands comprise sequences complementary to RNA of the
15 VEGF and/or VEGFr genes and wherein the sense strand sequences of the siNAs
comprise sequences identical or substantially similar to the sequences of the target
RNAs; and (b) introducing the siNA molecules into a cell under conditions suitable to
modulate the expression of the VEGF and/or VEGFr genes in the cell.

In another embodiment, the invention features a method for modulating the
20 expression of more than one VEGF and/or VEGFr gene within a cell comprising: (a)
synthesizing a siNA molecule of the invention, which can be chemically-modified,
wherein one of the siNA strands comprises a sequence complementary to RNA of the
VEGF and/or VEGFr gene and wherein the sense strand sequence of the siNA comprises
a sequence identical or substantially similar to the sequences of the target RNAs; and (b)
25 introducing the siNA molecule into a cell under conditions suitable to modulate the
expression of the VEGF and/or VEGFr genes in the cell.

In one embodiment, siNA molecules of the invention are used as reagents in ex
vivo applications. For example, siNA reagents are introduced into tissue or cells that are
transplanted into a subject for therapeutic effect. The cells and/or tissue can be derived
30 from an organism or subject that later receives the explant, or can be derived from
another organism or subject prior to transplantation. The siNA molecules can be used to

modulate the expression of one or more genes in the cells or tissue, such that the cells or tissue obtain a desired phenotype or are able to perform a function when transplanted in vivo. In one embodiment, certain target cells from a patient are extracted. These extracted cells are contacted with siRNAs targeting a specific nucleotide sequence within

5 the cells under conditions suitable for uptake of the siRNAs by these cells (e.g. using delivery reagents such as cationic lipids, liposomes and the like or using techniques such as electroporation to facilitate the delivery of siRNAs into cells). The cells are then reintroduced back into the same patient or other patients. In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene

10 in a tissue explant comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically-modified, wherein one of the siRNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siRNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the

15 tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in that organism.

In one embodiment, the invention features a method of modulating the expression

20 of a VEGF and/or VEGFr gene in a tissue explant comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically-modified, wherein one of the siRNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene and wherein the sense strand sequence of the siRNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siRNA

25 molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or

30 VEGFr gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in a tissue explant comprising:

(a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr genes; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate 5 the expression of the VEGF and/or VEGFr genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in that organism.

In one embodiment, the invention features a method of modulating the expression 10 of a VEGF and/or VEGFr gene in an organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecule into the organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the organism. The level of 15 VEGF or VEGFr can be determined as is known in the art or as described in Pavco USSN 10/438,493, incorporated by reference herein in its entirety including the drawings.

In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in an organism comprising: (a) 20 synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr genes; and (b) introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the organism. The level of VEGF or VEGFr can be determined as is known in the art 25 or as described in Pavco USSN 10/438,493, incorporated by reference herein in its entirety including the drawings.

In one embodiment, the invention features a method for modulating the expression of a VEGF and/or VEGFr gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA 30 comprises a single stranded sequence having complementarity to RNA of the VEGF

and/or VEGFr gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one VEGF and/or VEGFr gene within a cell comprising: (a) 5 synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) contacting the cell in vitro or in vivo with the siNA molecule under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the cell.

10 In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) contacting the cell of the tissue explant derived from a 15 particular organism with the siNA molecule under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in that organism.

20 In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecules into a cell of 25 the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in that organism.

30 In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene in an organism comprising: (a) synthesizing a siNA

molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecule into the organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the

5 organism.

In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in an organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the organism.

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In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene in an organism comprising contacting the organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the organism.

15

In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in an organism comprising contacting the organism with one or more siNA molecules of the invention under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the organism.

20

The siNA molecules of the invention can be designed to down regulate or inhibit target (VEGF and/or VEGFr) gene expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcripts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an

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alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the 5 protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented using known gene 10 sequences or from partial sequences available from an expressed sequence tag (EST).

In another embodiment, the siNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as VEGF and/or VEGFr family genes. As such, siNA molecules targeting multiple VEGF and/or VEGFr targets can provide increased therapeutic effect. In addition, siNA can be used to 15 characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward 20 pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example, the progression and/or maintenance of cancer.

In one embodiment, siNA molecule(s) and/or methods of the invention are used to down regulate the expression of gene(s) that encode RNA referred to by Genbank Accession, for example VEGF and/or VEGFr genes encoding RNA sequence(s) referred 25 to herein by Genbank Accession number, for example, Genbank Accession Nos. shown in **Table I**.

In one embodiment, the invention features a method comprising: (a) generating a library of siNA constructs having a predetermined complexity; and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within 30 the target RNA sequence. In one embodiment, the siNA molecules of (a) have strands of a fixed length, for example, about 23 nucleotides in length. In another embodiment, the

siNA molecules of (a) are of differing length, for example having strands of about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In one embodiment, the invention features a method comprising: (a) generating a randomized library of siNA constructs having a predetermined complexity, such as of 4^N , where N represents the number of base paired nucleotides in each of the siNA construct strands (e.g. for a siNA construct having 21 nucleotide sense and antisense strands with 19 base pairs, the complexity would be 4^{19}); and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target VEGF and/or VEGFr RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described in Example 7 herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of VEGF and/or VEGFr RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target VEGF and/or VEGFr RNA sequence. The target VEGF and/or VEGFr RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

30 In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA

of (a); and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In another embodiment, the siNA molecules of (b) are of differing length, for example 5 having strands of about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern 10 blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by expression in *in vivo* systems.

By "target site" is meant a sequence within a target RNA that is "targeted" for 15 cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

By "detectable level of cleavage" is meant cleavage of target RNA (and formation of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of 20 cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a 25 pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for diagnosing a disease or condition in a subject comprising administering to the subject a composition of the invention under conditions suitable for the diagnosis of the disease or 30 condition in the subject. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the

subject a composition of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds. In yet another embodiment, the invention features a method for reducing or preventing tissue rejection in a subject comprising administering 5 to the subject a composition of the invention under conditions suitable for the reduction or prevention of tissue rejection in the subject.

In another embodiment, the invention features a method for validating a VEGF and/or VEGFr gene target, comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes 10 a sequence complementary to RNA of a VEGF and/or VEGFr target gene; (b) introducing the siNA molecule into a cell, tissue, or organism under conditions suitable for modulating expression of the VEGF and/or VEGFr target gene in the cell, tissue, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, or organism.

15 In another embodiment, the invention features a method for validating a VEGF and/or VEGFr target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a VEGF and/or VEGFr target gene; (b) introducing the siNA molecule into a biological system under conditions suitable for modulating expression of 20 the VEGF and/or VEGFr target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

By "biological system" is meant, material, in a purified or unpurified form, from biological sources, including but not limited to human, animal, plant, insect, bacterial, viral or other sources, wherein the system comprises the components required for RNAi 25 activity. The term "biological system" includes, for example, a cell, tissue, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems that can be used in an *in vitro* setting.

By "phenotypic change" is meant any detectable change to a cell that occurs in response to contact or treatment with a nucleic acid molecule of the invention (e.g., 30 siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or

chemical changes as can be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Fluorescent Protein (GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

5 In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a VEGF and/or VEGFr target gene in a biological system, including, for example, in a cell, tissue, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one VEGF and/or VEGFr target gene in a biological system, including, for example, in a cell, tissue, or organism.

10 In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, 15 the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

15 In one embodiment, the synthesis of a siNA molecule of the invention, which can be chemically-modified, comprises: (a) synthesis of two complementary strands of the siNA molecule; (b) annealing the two complementary strands together under conditions 20 suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase tandem oligonucleotide synthesis.

20 In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, 25 wherein the second oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under

conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example 5 under hydrolysis conditions using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand 10 can comprise similar reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as 15 described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic conditions.

In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are 20 synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

In another embodiment, the invention features a method for synthesizing a siNA 25 duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other 30 strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second

oligonucleotide sequence strand under conditions suitable for isolating the full-length sequence comprising both siNA oligonucleotide strands connected by the cleavable linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one embodiment, cleavage of the linker molecule

5 in (c) above takes place during deprotection of the oligonucleotide, for example under hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c) above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a

10 cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that

15 can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is

20 complementary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the deprotection results in the cleavage of the linker joining the two oligonucleotide

25 sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

In another embodiment, the method of synthesis of siNA molecules of the invention comprises the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 30 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of Formulae I-VII or any combination thereof that increases the nuclease resistance of the
5 siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA
10 molecules having increased nuclease resistance.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siNA construct.

15 In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding
20 affinity between the sense and antisense strands of the siNA molecule.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within
25 a cell.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within
30 a cell.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to a chemically-modified siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA molecule.

In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against a VEGF and/or VEGFr in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA

molecule, DNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against VEGF and/or VEGFr comprising (a) 5 introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a VEGF and/or VEGFr target RNA 10 comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target RNA.

In yet another embodiment, the invention features a method for generating siNA 15 molecules with improved RNAi activity against a VEGF and/or VEGFr target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target DNA.

20 In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct.

In another embodiment, the invention features a method for generating siNA 25 molecules against VEGF and/or VEGFr with improved cellular uptake comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

30 In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more

chemical modifications described herein that increases the bioavailability of the siNA construct, for example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siNA construct, or by attaching conjugates that target specific tissue types or cell types *in vivo*. Non-limiting 5 examples of such conjugates are described in Vargeese *et al.*, U.S. Serial No. 10/201,394 incorporated by reference herein.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability, comprising (a) introducing a conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of 10 step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as 15 polyethyleneglycol (PEG); phospholipids; cholesterol; polyamines, such as spermine or spermidine; and others.

The term “ligand” refers to any compound or molecule, such as a drug, peptide, hormone, or neurotransmitter, that is capable of interacting with another compound, such as a receptor, either directly or indirectly. The receptor that interacts with a ligand can be 20 present on the surface of a cell or can alternately be an intercellular receptor. Interaction of the ligand with the receptor can result in a biochemical reaction, or can simply be a physical interaction or association.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing an 25 excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, nanoparticles, receptors, ligands, and others.

In another embodiment, the invention features a method for generating siNA 30 molecules of the invention with improved bioavailability comprising (a) introducing nucleotides having any of Formulae I-VII or any combination thereof into a siNA

molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 2,000 to about 50,000 daltons (Da).

The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into 10 cells of interest as described herein (e.g., using lipids and other methods of transfection known in the art, see for example Beigelman *et al.*, US 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug optimization, and in drug discovery (see for example Usman *et al.*, USSN 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

15 The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression or viral replication, for example by mediating RNA interference "RNAi" or 20 gene silencing in a sequence-specific manner; see for example Bass, 2001, *Nature*, 411, 428-429; Elbashir *et al.*, 2001, *Nature*, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; 25 Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237; Hutvagner and Zamore, 2002, *Science*, 297, 2056-60; McManus *et al.*, 30 2002, *RNA*, 8, 842-850; Reinhart *et al.*, 2002, *Gene & Dev.*, 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). Non limiting examples of siNA molecules of the

invention are shown in **Figures 4-6, and Tables II, III, and IV** herein. For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19 base pairs); the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide sequence corresponding to the

target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez *et al.*, 2002, *Cell.*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiment, the siNA

5 molecule of the invention comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic intercations, and/or stacking interactions. In certain embodiments, the siNA molecules

10 of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified

15 nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules of the invention optionally do not

20 include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30,

25 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded

30 RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other

terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic regulation of 5 gene expression by siNA molecules of the invention can result from siNA mediated modification of chromatin structure to alter gene expression (see, for example, Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237).

By “asymmetric hairpin” as used herein is meant a linear siNA molecule 10 comprising an antisense region, a loop portion that can comprise nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin siNA molecule of the invention can comprise an antisense region having length 15 sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22 (e.g., about 19, 20, 21, or 22) nucleotides) and a loop region comprising about 4 to about 8 (e.g., about 4, 5, 6, 7, or 8) nucleotides, and a sense region having about 3 to about 18 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) nucleotides that are complementary to the antisense region. The asymmetric hairpin siNA molecule can also 20 comprise a 5'-terminal phosphate group that can be chemically modified. The loop portion of the asymmetric hairpin siNA molecule can comprise nucleotides, non-nucleotides, linker molecules, or conjugate molecules as described herein.

By “asymmetric duplex” as used herein is meant a siNA molecule having two 25 separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22 (e.g. about 19, 20, 21, or 22) nucleotides) and a 30 sense region having about 3 to about 18 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) nucleotides that are complementary to the antisense region.

By "modulate" is meant that the expression of the gene, or level of RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the 5 absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is 10 reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA 15 molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence.

By "gene" or "target gene" is meant, a nucleic acid that encodes an RNA, for 20 example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, 25 virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts.

By "VEGF" as used herein is meant, any vascular endothelial growth factor (e.g., VEGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D) protein, peptide, or polypeptide having 30 vascular endothelial growth factor activity, such as encoded by VEGF Genbank Accession Nos. shown in **Table I**. The term VEGF also refers to nucleic acid sequences

enclosing any vascular endothelial growth factor protein, peptide, or polypeptide having vascular endothelial growth factor activity.

5 By “VEGF-B” is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_003377, having vascular endothelial growth factor type B activity. The term VEGF-B also refers to nucleic acid sequences enclosing any VEGF-B protein, peptide, or polypeptide having VEGF-B activity.

10 By “VEGF-C” is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_005429, having vascular endothelial growth factor type C activity. The term VEGF-C also refers to nucleic acid sequences enclosing any VEGF-C protein, peptide, or polypeptide having VEGF-C activity.

15 By “VEGF-D” is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_004469, having vascular endothelial growth factor type D activity. The term VEGF-D also refers to nucleic acid sequences enclosing any VEGF-D protein, peptide, or polypeptide having VEGF-D activity.

20 By “VEGFr” as used herein is meant, any vascular endothelial growth factor receptor protein, peptide, or polypeptide (e.g., VEGFr1, VEGFr2, or VEGFr3, including both membrane bound and/or soluble forms thereof) having vascular endothelial growth factor receptor activity, such as encoded by VEGFr Genbank Accession Nos. shown in **Table I**. The term VEGFr also refers to nucleic acid sequences enclosing any vascular endothelial growth factor receptor protein, peptide, or polypeptide having vascular endothelial growth factor receptor activity.

25 By “VEGFr1” is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_002019, having vascular endothelial growth factor receptor type 1 (*flt*) activity, for example, having the ability to bind a vascular endothelial growth factor. The term VEGF1 also refers to nucleic acid sequences enclosing any VEGFr1 protein, peptide, or polypeptide having VEGFr1 activity.

By "VEGFr2" is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_002253, having vascular endothelial growth factor receptor type 2 (*kdr*) activity, for example, having the ability to bind a vascular endothelial growth factor. The term VEGF2 also refers to nucleic acid sequences encoding any VEGFr2 protein, peptide, or polypeptide having VEGFr2 activity.

By "VEGFr3" is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_002020 having vascular endothelial growth factor receptor type 3 (*kdr*) activity, for example, having the ability to bind a vascular endothelial growth factor. The term VEGF3 also refers to nucleic acid sequences encoding any VEGFr3 protein, peptide, or polypeptide having VEGFr3 activity.

By "highly conserved sequence region" is meant, a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

By "sense region" is meant a nucleotide sequence of a siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence.

By "antisense region" is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule.

By "target nucleic acid" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity.

Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner *et al.*, 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.*, 1986, *Proc. Nat. Acad. Sci. USA* 83:9373-9377; Turner *et al.*, 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, or 10 nucleotides out of a total of 10 nucleotides in the first oligonucleotide being paired to a second nucleic acid sequence having 10 nucleotides represents 50%, 60%, 70%, 80%, 90%, and 100% complementary respectively). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

The siRNA molecules of the invention represent a novel therapeutic approach to treat a variety of pathologic indications or other conditions, such as tumor angiogenesis and cancer, including but not limited to breast cancer, lung cancer (including non-small cell lung carcinoma), prostate cancer, colorectal cancer, brain cancer, esophageal cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, skin cancers, nasopharyngeal carcinoma, liposarcoma, epithelial carcinoma, renal cell carcinoma, gallbladder adeno carcinoma, parotid adenocarcinoma, ovarian cancer, melanoma, lymphoma, glioma, endometrial sarcoma, multidrug resistant cancers, diabetic retinopathy, macular degeneration, neovascular glaucoma, myopic degeneration, arthritis, psoriasis, endometriosis, female reproduction, verruca vulgaris, angiofibroma of tuberous sclerosis, port-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber syndrome, Osler-Weber-Rendu syndrome, renal disease such as Autosomal dominant polycystic kidney disease (ADPKD), and any other diseases or conditions that are related to or will respond to the levels of VEGF, VEGFr1, VEGFr2 and/or VEGFr3 in a cell or tissue, alone or in combination with other therapies. The reduction of VEGF, VEGFr1, VEGFr2 and/or VEGFr3 expression (specifically VEGF, VEGFr1, VEGFr2 and/or VEGFr3 gene RNA levels) and thus reduction in the level of the respective protein relieves, to some extent, the symptoms of the disease or condition.

In one embodiment of the present invention, each sequence of a siRNA molecule of the invention is independently about 18 to about 24 nucleotides in length, in specific embodiments about 18, 19, 20, 21, 22, 23, or 24 nucleotides in length. In another

embodiment, the siNA duplexes of the invention independently comprise about 17 to about 23 base pairs (e.g., about 17, 18, 19, 20, 21, 22 or 23). In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (e.g., about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44 (e.g., 38, 39, 40, 41, 42, 43 or 44) nucleotides in length and comprising about 16 to about 22 (e.g., about 16, 17, 18, 19, 20, 21 or 22) base pairs. Exemplary siNA molecules of the invention are shown in **Table II**. Exemplary synthetic siNA molecules of the invention are shown in **Tables III and IV** and/or **Figures 4-5**.

As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, infusion pump or stent, with or without their incorporation in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in **Tables II-III** and/or **Figures 4-5**. Examples of such nucleic acid molecules consist essentially of sequences defined in these tables and figures. Furthermore, the chemically modified constructs described in **Table IV** can be applied to any siNA sequence of the invention.

In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β -D-ribo-furanose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA,

recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siRNA or internally, for example at one or more nucleotides of the RNA.

5 Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

By "subject" is meant an organism, which is a donor or recipient of explanted cells
 10 or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. A subject can be a mammal or mammalian cells, including a human or human cells.

The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term
 15 phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

The term "phosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise an acetyl or protected acetyl group.

The term "thiophosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z comprises an acetyl or protected acetyl group and W comprises a sulfur atom or alternately W comprises an acetyl or protected acetyl group and Z comprises a sulfur atom.

The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination
 25 between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research*, 29, 2437-2447).

The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

5 The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed herein (e.g., cancers and other proliferative conditions). For example, to treat a particular disease or condition, the siNA molecules can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

10 In a further embodiment, the siNA molecules can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described molecules could be used in combination with one or more known therapeutic agents to treat a disease or condition. Non-limiting examples of other therapeutic agents that can be readily combined with a siNA molecule of the invention are enzymatic
15 nucleic acid molecules, allosteric nucleic acid molecules, antisense, decoy, or aptamer nucleic acid molecules, antibodies such as monoclonal antibodies, small molecules, and other organic and/or inorganic compounds including metals, salts and ions.

20 In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention, in a manner which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such expression vectors are described in Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505;
25 Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725.

In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown in **Table I**.

5 In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be 10 DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules. Such vectors can be repeatedly 15 administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired 20 target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable 30 linker used for solid phase synthesis on a solid support. The synthesis can be either solid

phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to 5 form a siNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

10 **Figure 2** shows a MALDI-TOF mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

15 **Figure 3** shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be introduced directly into a cell by appropriate means. An active siNA complex forms 20 which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

25 **Figure 4A-F** shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N). Various modifications are shown for the sense and antisense strands of the siNA constructs.

30 **Figure 4A:** The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides,

deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all nucleotides present are ribonucleotides except for 5 (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s" connects the (N N) nucleotides in the antisense strand.

10 **Figure 4B:** The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical 15 modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides 20 except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s" connects the (N N) nucleotides in the sense and antisense strand.

25 **Figure 4C:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications 30 described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that

may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s" connects the (N N) nucleotides in the antisense strand.

Figure 4D: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s" connects the (N N) nucleotides in the antisense strand.

Figure 4E: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate,

phosphorodithioate or other modified internucleotide linkage as described herein, shown as “s” connects the (N N) nucleotides in the antisense strand.

Figure 4F: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and 5 wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The 10 antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, 15 deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as “s” connects the (N N) nucleotides in the antisense strand. The antisense strand of constructs A-F comprise sequence complementary to any target nucleic acid sequence of the invention. 20 Furthermore, when a glyceryl moiety (L) is present at the 3'-end of the antisense strand for any construct shown in Figure 4 A-F, the modified internucleotide linkage is optional.

Figure 5A-F shows non-limiting examples of specific chemically-modified siNA sequences of the invention. **A-F** applies the chemical modifications described in **Figure 4A-F** to a VEGFr2 siNA sequence. Such chemical modifications can be applied to any 25 sequence herein, such as any VEGF, VEGFr1, VEGFr2, or VEGFr3 sequence.

Figure 6 shows non-limiting examples of different siNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs 30 described herein. Bracketed regions represent nucleotide overhangs, for example comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides.

Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 *in vivo* and/or 5 *in vitro*. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 *in vivo* and/or *in vitro*, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 *in vivo* and/or *in vitro*. As such, the stability and/or activity of the siNA constructs can be modulated based on the design of the siNA construct for use 10 *in vivo* or *in vitro* and/or *in vitro*.

Figure 7A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

15 **Figure 7A:** A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siNA) to a predetermined VEGF and/or VEGFr target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.

20 **Figure 7B:** The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence that will result in a siNA transcript having specificity for a VEGF and/or VEGFr target sequence and having self-complementary sense and antisense regions.

25 **Figure 7C:** The construct is heated (for example to about 95°C) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-terminal nucleotide overhang results from the transcription, for example by engineering restriction sites and/or utilizing a poly-U termination region as described in Paul *et al.*, 2002, *Nature Biotechnology*, 29, 505-508.

Figure 8A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

5 **Figure 8A:** A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence followed by a region having sequence identical (sense region of siNA) to a predetermined VEGF and/or VEGFr target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X).

10 **Figure 8B:** The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence.

15 **Figure 8C:** The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

Figure 9A-E is a diagrammatic representation of a method used to determine target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

20 **Figure 9A:** A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

25 **Figure 9B&C:** (**Figure 9B**) The sequences are pooled and are inserted into vectors such that (**Figure 9C**) transfection of a vector into cells results in the expression of the siNA.

Figure 9D: Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

Figure 9E: The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

Figure 10 shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including (1) [3-3']-inverted deoxyribose; (2) deoxyribonucleotide; (3) [5'-3']-3'-deoxyribonucleotide; (4) [5'-3']-ribonucleotide; (5) [5'-3']-3'-O-methyl ribonucleotide; (6) 3'-glyceryl; (7) [3'-5']-3'-deoxyribonucleotide; (8) [3'-3']-deoxyribonucleotide; (9) [5'-2']-deoxyribonucleotide; and (10) [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

Figure 11 shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistance while preserving the ability to mediate RNAi activity. Chemical modifications are introduced into the siNA construct based on educated design parameters (e.g. introducing 2'-mofications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct in tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

Figure 12 shows a non-limiting example of siNA mediated inhibition of VEGF-induced angiogenesis using the rat corneal model of angiogenesis. siNA targeting site 2340 of VEGFr1 RNA (shown as RPI No. 29695/29699 sense strand/antisense strand) was compared to an inverted control siNA (shown as RPI No. 29983/29984 sense strand/antisense strand) at three different concentrations (1ug, 3ug, and 10ug) and

compared to a VEGF control in which no siNA was administered. As shown in the Figure, siNA constructs targeting VEGFr1 RNA can provide significant inhibition of angiogenesis in the rat corneal model.

5 **Figure 13** shows a non-limiting example of reduction of VEGFr1 mRNA in A375 cells mediated by chemically-modified siNAs that target VEGFr1 mRNA. A549 cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. A screen of siNA constructs (Stabilization “Stab” chemistries are shown in **Table IV**, constructs are referred to by RPI number, see **Table III**) comprising Stab 4/5 chemistry (RPI 31190/31193), Stab 1/2 chemistry (RPI 31183/31186 and RPI 31184/31187), and 10 unmodified RNA (RPI 30075/30076) were compared to untreated cells, matched chemistry inverted control siNA constructs, (RPI 31208/31211, RPI 31201/31204, RPI 31202/31205, and RPI 30077/30078) scrambled siNA control constructs (Scram1 and Scram2), and cells transfected with lipid alone (transfection control). All of the siNA constructs show significant reduction of VEGFr1 RNA expression.

15 **Figure 14** shows non-limiting examples of phosphorylated siNA molecules of the invention, including linear and duplex constructs and asymmetric derivatives thereof.

Figure 15 shows non-limiting examples of chemically modified terminal phosphate groups of the invention.

20 **Figure 16** shows a non-limiting example of inhibition of VEGF induced neovascularization in the rat corneal model. VEGFr1 site 349 active siNA having “Stab 9/10” chemistry (Compound No. 31270/31273) was tested for inhibition of VEGF-induced angiogenesis at three different concentrations (2.0 ug, 1.0 ug, and 0.1 ug dose response) as compared to a matched chemistry inverted control siNA construct (Compound No. 31276/31279) at each concentration and a VEGF control in which no 25 siNA was administered. As shown in the figure, the active siNA construct having “Stab 9/10” chemistry (Compound No. 31270/31273) is highly effective in inhibiting VEGF-induced angiogenesis in the rat corneal model compared to the matched chemistry inverted control siNA at concentrations from 0.1 ug to 2.0 ug.

30 **Figure 17** shows a non-limiting example of inhibition of VEGF induced neovascularization in a mouse model of coroidal neovascularization via intraocular

administration of siNA. VEGFr1 site 349 active siNA having “Stab 9/10” chemistry (Compound No. 31270/31273) was tested for inhibition of neovascularization at two different concentrations (1.5 ug, and 0.5 ug) as compared to a matched chemistry inverted control siNA construct (Compound No. 31276/31279) and phosphate buffered saline (PBS). siNA constructs were administered intraocularly on days 1 and 7 following laser induced injury to the choroid, and choroidal neovascularization assessed on day 14. As shown in the figure, the active siNA construct having “Stab 9/10” chemistry (Compound No. 31270/31273) is highly effective in inhibiting neovascularization via intraocular administration in this model.

10 **Figure 18** shows a non-limiting example of inhibition of VEGF induced neovascularization in a mouse model of coroidal neovascularization via periocular administration of siNA. VEGFr1 site 349 active siNA having “Stab 9/10” chemistry (Compound No. 31270/31273) was tested for inhibition of neovascularization at two different concentrations (1.5 ug with a saline control, and 0.5 ug with an inverted siNA 15 control, Compound No. 31276/31279). Eight mice were used in each arm of the study with one eye receiving the active siNA and the other eye receiving the saline or inverted control. siNA constructs and controls were administered daily up to 14 days, and neovascularization was assessed at day 17 following laser induced injury to the choroid. As shown in the figure, the active siNA construct having “Stab 9/10” chemistry 20 (Compound No. 31270/31273) is highly effective in inhibiting neovascularization via periocular administration in this model.

25 **Figure 19** shows another non-limiting example of inhibition of VEGF induced neovascularization in a mouse model of coroidal neovascularization via periocular administration of siNA. VEGFr1 site 349 active siNA having “Stab 9/10” chemistry (Compound No. 31270/31273) was tested for inhibition of neovascularization at two 30 different concentrations (1.5 ug with an inverted siNA control, Compound No. 31276/31279 and 0.5 ug with a saline control). Nine mice were used in the active versus inverted arm of the study with one eye receiving the active siNA and the other eye receiving the inverted control. Eight mice were used in the active versus saline arm of the study with one eye receiving the active siNA and the other eye receiving the saline control. siNA constructs and controls were administered daily up to 14 days, and neovascularization was assessed at day 17 following laser induced injury to the choroid.

As shown in the figure, the active siNA construct having "Stab 9/10" chemistry (Compound No. 31270/31273) is highly effective in inhibiting neovascularization via periocular administration in this model.

DETAILED DESCRIPTION OF THE INVENTION

5 Mechanism of action of Nucleic Acid Molecules of the Invention

The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that chemically-modified short interfering nucleic acids possess similar or improved capacity 10 to mediate RNAi as do siRNA molecules and are expected to possess improved stability and activity *in vivo*; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured *in vitro* and/or *in vivo* where the RNAi activity is a reflection of both the ability of the siNA to mediate 15 RNAi and the stability of the siNAs of the invention. In this invention, the product of these activities can be increased *in vitro* and/or *in vivo* compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the siNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced *in vitro* and/or *in vivo*.

20 RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an 25 evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a 30 cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a

mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

5 The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes.

10 Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded

15 RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). In addition, RNA interference can also involve small RNA (e.g., micro-RNA or miRNA) mediated gene silencing, presumably through cellular mechanisms that regulate chromatin structure and thereby

20 prevent transcription of target gene sequences (see for example Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237). As such, siRNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein

25 such interaction results in gene silencing either at the transcriptional level or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 30 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human

embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two 2-nucleotide 3'-
 5 terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in
 10 the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of
 15 siRNA constructs may occur *in vivo*.

Synthesis of Nucleic acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this
 20 invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid
 25 to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-
 30 19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No.

6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. 5 synthesizer using a 0.2 μ mol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 second coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μ mol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by 10 Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μ L of 0.11 M = 6.6 μ mol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μ L of 0.25 M = 15 μ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μ L of 0.11 M = 4.4 μ mol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 15 μ L of 0.25 M = 10 μ mol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in 20 methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I₂, 49 mM pyridine, 9% water in THF (PERSEPTIVETM). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from 25 American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

30 Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aqueous methylamine (1 mL) at 65 °C for 10 minutes. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H₂O/3:1:1, vortexed and

the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μ mol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μ mol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μ L of 0.11 M = 6.6 μ mol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μ L of 0.25 M = 15 μ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μ L of 0.11 M = 13.2 μ mol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μ L of 0.25 M = 30 μ mol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I_2 , 49 mM pyridine, 9% water in THF (PERSEPTIVETM). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide 0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the 5 polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 µL of a solution of 1.5 mL N-methylpyrrolidinone, 750 µL 10 TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH₄HCO₃.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 minutes. 15 The vial is brought to room temperature TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 minutes. The sample is cooled at –20 °C and then quenched with 1.5 M NH₄HCO₃.

For purification of the trityl-on oligomers, the quenched NH₄HCO₃ solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile 20 followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 minutes. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 25 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation 30 (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No.

WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate siNA fragments or strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the RNA molecule.

The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by gel electrophoresis using general methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see e.g., Eckstein *et al.*, International Publication No. WO 5 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold *et al.*, U.S. Pat. No. 6,300,074; and Burgin *et al.*, *supra*; all of which are incorporated by reference herein). All of the 10 above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

15 There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al.*, International Publication PCT No. WO 92/07065; Perrault *et al.* *Nature*, 1990, 344, 565-568; Pieken *et al.* *Science*, 1991, 20 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.*, 1992, 17, 334-339; Usman *et al.* International Publication PCT No. WO 93/15187; Sproat, U.S. Pat. No. 5,334,711 and Beigelman *et al.*, 1995, *J. Biol. Chem.*, 270, 25702; Beigelman *et al.*, International PCT publication No. WO 97/26270; Beigelman *et al.*, U.S. Pat. No. 5,716,824; Usman *et al.*, U.S. Pat. No. 5,627,053; Woolf *et al.*, International PCT 25 30 Publication No. WO 98/13526; Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.*, 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu.*

Rev. Biochem., 67, 99-134; and Burlina *et al.*, 1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid 5 molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi in cells is not significantly inhibited.

While chemical modification of oligonucleotide internucleotide linkages with 10 phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these 15 molecules.

Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the 20 goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, 25 *Methods in Enzymology* 211,3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp 30 nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine

within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.*, International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of siNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, cholesterol, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA

molecule of the invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability of a nucleic acid-based biodegradable linker molecule can be modulated by using various
5 chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer
10 nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological
15 system, for example enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein, refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include
20 therapeutically active molecules such as antibodies, cholesterol, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention
25 also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a
30 phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

Therapeutic nucleic acid molecules (e.g., siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular 5 therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

In yet another embodiment, siNA molecules having chemical modifications that 10 maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered.

Use of the nucleic acid-based molecules of the invention will lead to better treatment of the disease progression by affording the possibility of combination therapies 15 (e.g., multiple siNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment of subjects with siNA molecules can also include combinations of different types of nucleic acid molecules, such as enzymatic nucleic acid 20 molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and aptamers.

In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'- cap structure, for example on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

25 By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic *et al.*, U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'- 30 terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap includes, but is not limited to, glyceryl, inverted deoxy abasic residue (moiety); 4',5'-

methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety.

10 Non-limiting examples of the 3'-cap include, but are not limited to, glyceryl, inverted deoxy abasic residue (moiety), 4', 5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, 20 phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

25 By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

30 An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to

4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at

the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra*, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amide carbamate, carboxymethyl, acetamide, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties*, in *Modern Synthetic Methods*, VCH, 331-417, and Mesmaeker *et al.*, 1994, *Novel Backbone Replacements for Oligonucleotides*, in *Carbohydrate Modifications in Antisense Research*, ACS, 24-39.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic *et al.*, U.S. Pat. No. 5,998,203.

30 By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of β -D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

5 In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH₂ or 2'-O- NH₂, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.*, U.S. Pat. No. 5,672,695 and Matulic-Adamic *et al.*, U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

10 Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro*, stability, and ease of introduction of such oligonucleotides to the target site, *e.g.*, to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

15 Administration of Nucleic Acid Molecules

A siNA molecule of the invention can be adapted for use to treat, for example, tumor angiogenesis and cancer, including but not limited to breast cancer, lung cancer (including non-small cell lung carcinoma), prostate cancer, colorectal cancer, brain cancer, esophageal cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, skin cancers, nasopharyngeal carcinoma, liposarcoma, epithelial carcinoma, renal cell carcinoma, gallbladder adeno carcinoma, parotid adenocarcinoma, ovarian cancer, melanoma, lymphoma, glioma, endometrial sarcoma, multidrug resistant cancers, diabetic retinopathy, macular degeneration, neovascular glaucoma, myopic degeneration, arthritis, psoriasis, endometriosis, female reproduction, verruca vulgaris, angiofibroma of tuberous sclerosis, pot-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber syndrome, Osler-Weber-Rendu syndrome, renal disease such as Autosomal dominant polycystic kidney disease (ADPKD), and any other diseases or conditions that are related to or will respond to the levels of VEGF, VEGFr1, VEGFr2 and/or VEGFr3 in a cell or tissue, alone or in combination with other therapies. For example, a siNA molecule can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable

formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee 5 *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-192, all of which are incorporated herein by reference. Beigelman *et al.*, U.S. Pat. No. 6,395,713 and Sullivan *et al.*, PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to 10 those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as biodegradable polymers, hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-1074; Wang *et al.*, International PCT publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLCA microspheres (see for 15 example US Patent 6,447,796 and US Patent Application Publication No. US 2002130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). Alternatively, the nucleic acid/vehicle combination is locally delivered by 20 direct injection or by use of an infusion pump. Direct injection of the nucleic acid molecules of the invention, whether subcutaneous, intramuscular, or intradermal, can take place using standard needle and syringe methodologies, or by needle-free technologies such as those described in Conry *et al.*, 1999, *Clin. Cancer Res.*, 5, 2330-2337 and Barry *et al.*, International PCT Publication No. WO 99/31262. The molecules 25 of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, modulate the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a subject.

In one embodiment, a compound, molecule, or composition for the treatment of ocular conditions (e.g., macular degeneration, diabetic retinopathy etc.) is administered to a subject intraocularly or by intraocular means. In another embodiment, a compound, 30 molecule, or composition for the treatment of ocular conditions (e.g., macular degeneration, diabetic retinopathy etc.) is administered to a subject periocularly or by periocular means (see for example Ahlheim *et al.*, International PCT publication No. WO 03/24420). In one embodiment, a siNA molecule and/or formulation or composition

thereof is administered to a subject intraocularly or by intraocular means. In another embodiment, a siNA molecule and/or formualtion or composition thereof is administered to a subject periocularly or by periocular means. Periocular administration generally provides a less invasive approach to administering siNA molecules and formualtion or composition thereof to a subject (see for example Ahlheim et al., International PCT publication No. WO 03/24420). The use of periocular administration also minimizes the risk of retinal detachment, allows for more frequent dosing or administration, provides a clinically relevant route of administration for macular degeneration and other optic conditions, and also provides the possiblity of using resevoirs (e.g., implants, 10 pumps or other devices) for drug delivery.

In one embodiment, a siNA molecule of the invention is complexed with membrane disruptive agents such as those described in U.S. Patent Appliaction Publication No. 20010007666, incorporated by reference herein in its entirety including the drawings. In another embodiment, the membrane disruptive agent or agents and the 15 siNA molecule are also complexed with a cationic lipid or helper lipid molecule, such as those lipids described in U.S. Patent No. 6,235,310, incorporated by reference herein in its entirety including the drawings.

Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and 20 the like. The polynucleotides of the invention can be administered (e.g., RNA, DNA or protein) and introduced into a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as 25 tablets, capsules or elixirs for oral administration, suppositories for rectal administration, sterile solutions, suspensions for injectable administration, and the other compositions known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, 30 e.g., acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms 5 should not prevent the composition or formulation from reaching a target cell (*i.e.*, a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

10 By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the 15 invention to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the 20 association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as cells producing excess VEGF and/or VEGFr.

25 By "pharmaceutically acceptable formulation" is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85), which can enhance entry of drugs into the CNS (Jolliet-Riant and Tillement, 1999, *Fundam. Clin. 30 Pharmacol.*, 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after intracerebral implantation (Emerich, DF *et al*, 1999, *Cell Transplant*, 8, 47-58) (Alkermes, Inc. Cambridge, MA); and loaded

nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (*Prog Neuropsychopharmacol Biol Psychiatry*, 23, 941-949, 1999). Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention 5 include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058.

The invention also features the use of the composition comprising surface-10 modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the 15 encapsulated drug (Lasic *et al. Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al., Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al., Science* 1995, 267, 1275-1276; Oku *et al.*, 1995, *Biochim. Biophys. Acta*, 1238, 86-90). The long-circulating liposomes enhance the 20 pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al., J. Biol. Chem.* 1995, 42, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392). Long- 25 circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired 30 compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R.

Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

5 A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent 10 medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

15 The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a 20 pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, 25 lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

30 Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic

pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for 5 example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl 10 monostearate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

15 Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, 20 lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with 25 partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

30 Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral

oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid

5 Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring 10 agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, 15 lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, 20 propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. 25 The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any 30 bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local 10 anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending 15 upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, 20 and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate 25 quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the 30 beneficial effects while reducing the presence of side effects.

In one embodiment, the invention comprises compositions suitable for administering nucleic acid molecules of the invention to specific cell types. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal 5 glycoproteins, such as asialoorosomucoid (ASOR). In another example, the folate receptor is overexpressed in many cancer cells. Binding of such glycoproteins, synthetic glycoconjugates, or folates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triatennary structures are bound with greater affinity than biatennary or monoatennary 10 chains (Baenziger and Fiete, 1980, *Cell*, 22, 611-620; Connolly *et al.*, 1982, *J. Biol. Chem.*, 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.*, 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the receptor, compared to galactose. This "clustering effect" 15 has also been described for the binding and uptake of mannosyl-terminating glycoproteins or glycoconjugates (Ponpipom *et al.*, 1981, *J. Med. Chem.*, 24, 1388-1395). The use of galactose, galactosamine, or folate based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to, for example, the treatment of liver disease, cancers of the liver, or other cancers. The 20 use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavailability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention. Non-limiting examples of such bioconjugates are described in Vargeese *et al.*, USSN 10/201,394, filed August 13, 2001; and Matulic-Adamic *et al.*, USSN 10/151,116, filed May 17, 2002. In one embodiment, 25 nucleic acid molecules of the invention are complexed with or covalently attached to nanoparticles, such as Hepatitis B virus S, M, or L envelope proteins (see for example Yamado *et al.*, 2003, *Nature Biotechnology*, 21, 885). In one embodiment, nucleic acid molecules of the invention are delivered with specificity for human tumor cells, specifically non-apoptotic human tumor cells including for example T-cells, hepatocytes, 30 breast carcinoma cells, ovarian carcinoma cells, melanoma cells, intestinal epithelial cells, prostate cells, testicular cells, non-small cell lung cancers, small cell lung cancers, etc.

Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985, *Science*, 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci., USA* 83, 399; Scanlon *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Dropulic *et al.*, 1992, *J. Virol.*, 66, 1432-41; Weersasinghe *et al.*, 1991, *J. Virol.*, 65, 5531-4; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science*, 247, 1222-1225; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Good *et al.*, 1997, *Gene Therapy*, 4, 45. Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992, *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856.

In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based constructs are used to express nucleic acid molecules of the invention (see for example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant vectors capable of expressing the siNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the 5 siNA molecules of the instant invention can be operably linked in a manner that allows expression of the siNA molecule (see for example Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

10 In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (e.g., eukaryotic pol I, II or III initiation region); b) a transcription termination region (e.g., eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siNA molecules of the instant invention, wherein said sequence is operably linked to said initiation region and said 15 termination region in a manner that allows expression and/or delivery of the siNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

Transcription of the siNA molecule sequences can be driven from a promoter for 20 eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA 25 polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (e.g. 30 Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO*

J., 11, 4411-8; Lisziewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A.*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are 5 useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson *et al.*, *supra*; Couture and Stinchcomb, 1996, *supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, U.S. Pat. No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736. The above siNA transcription units can be incorporated into a variety of vectors for 10 introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, *supra*).

15 In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siNA molecule, wherein the sequence is operably linked to the initiation region and the termination 20 region in a manner that allows expression and/or delivery of the siNA molecule.

25 In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is 30 operably linked to the initiation region, the intron and the termination region in a manner which allows expression and/or delivery of the nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and 5 wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

VEGF/VEGFr biology and biochemistry

The following discussion is adapted from R&D Systems, Cytokine Mini Reviews, 10 Vascular Endothelial Growth Factor (VEGF), Copyright ©2002 R&D Systems. Angiogenesis is a process of new blood vessel development from pre-existing vasculature. It plays an essential role in embryonic development, normal growth of tissues, wound healing, the female reproductive cycle (i.e., ovulation, menstruation and 15 placental development), as well as a major role in many diseases. Particular interest has focused on cancer, since tumors cannot grow beyond a few millimeters in size without 20 developing a new blood supply. Angiogenesis is also necessary for the spread and growth of tumor cell metastases.

One of the most important growth and survival factors for endothelium is vascular 25 endothelial growth factor (VEGF). VEGF induces angiogenesis and endothelial cell proliferation and plays an important role in regulating vasculogenesis. VEGF is a heparin-binding glycoprotein that is secreted as a homodimer of 45 kDa. Most types of cells, but usually not endothelial cells themselves, secrete VEGF. Since the initially discovered VEGF, VEGF-A, increases vascular permeability, it was known as vascular 30 permeability factor. In addition, VEGF causes vasodilatation, partly through stimulation of nitric oxide synthase in endothelial cells. VEGF can also stimulate cell migration and inhibit apoptosis.

There are several splice variants of VEGF-A. The major ones include: 121, 165, 189 and 206 amino acids (aa), each one comprising a specific exon addition. VEGF165 is the most predominant protein, but transcripts of VEGF 121 may be more abundant. 30 VEGF206 is rarely expressed and has been detected only in fetal liver. Recently, other splice variants of 145 and 183 aa have also been described. The 165, 189 and 206 aa

splice variants have heparin-binding domains, which help anchor them in extracellular matrix and are involved in binding to heparin sulfate and presentation to VEGF receptors. Such presentation is a key factor for VEGF potency (i.e., the heparin-binding forms are more active). Several other members of the VEGF family have been cloned

5 including VEGF-B, -C, and -D. Placenta growth factor (PIGF) is also closely related to VEGF-A. VEGF-A, -B, -C, -D, and PIGF are all distantly related to platelet-derived growth factors-A and -B. Less is known about the function and regulation of VEGF-B, -C, and -D, but they do not seem to be regulated by the major pathways that regulate VEGF-A.

10 VEGF-A transcription is potentiated in response to hypoxia and by activated oncogenes. The transcription factors, hypoxia inducible factor-1a (hif-1a) and -2a, are degraded by proteasomes in normoxia and stabilized in hypoxia. This pathway is dependent on the Von Hippel-Lindau gene product. Hif-1a and hif-2 a heterodimerize with the aryl hydrocarbon nuclear translocator in the nucleus and bind the VEGF promoter/enhancer. This is a key pathway expressed in most types of cells. Hypoxia inducibility, in particular, characterizes VEGF-A versus other members of the VEGF family and other angiogenic factors. VEGF transcription in normoxia is activated by many oncogenes, including H-ras and several transmembrane tyrosine kinases, such as the epidermal growth factor receptor and erbB2. These pathways together account for a

15 marked upregulation of VEGF-A in tumors compared to normal tissues and are often of prognostic importance.

20

There are three receptors in the VEGF receptor family. They have the common properties of multiple IgG-like extracellular domains and tyrosine kinase activity. The enzyme domains of VEGF receptor 1 (VEGFr1, also known as Flt-1), VEGFr2 (also known as KDR or Flk-1), and VEGFr3 (also known as Flt-4) are divided by an inserted sequence. Endothelial cells also express additional VEGF receptors, Neuropilin-1 and Neuropilin-2. VEGF-A binds to VEGFr1 and VEGFr2 and to Neuropilin-1 and Neuropilin-2. PIGF and VEGF-B bind VEGFr1 and Neuropilin-1. VEGF-C and -D bind VEGFr3 and VEGFr2.

30 The VEGF-C/VEGFr3 pathway is important for lymphatic proliferation. VEGFr3 is specifically expressed on lymphatic endothelium. A soluble form of Flt-1 can be

detected in peripheral blood and is a high affinity ligand for VEGF. Soluble Flt-1 can be used to antagonize VEGF function. VEGFr1 and VEGFr2 are upregulated in tumor and proliferating endothelium, partly by hypoxia and also in response to VEGF-A itself. VEGFr1 and VEGFr2 can interact with multiple downstream signaling pathways via proteins such as PLC-g, Ras, Shc, Nck, PKC and PI3-kinase. VEGFr1 is of higher affinity than VEGFr2 and mediates motility and vascular permeability. VEGFr2 is necessary for proliferation.

VEGF can be detected in both plasma and serum samples of patients, with much higher levels in serum. Platelets release VEGF upon aggregation and may be a major 10 source of VEGF delivery to tumors. Several studies have shown that association of high serum levels of VEGF with poor prognosis in cancer patients may be correlated with an elevated platelet count. Many tumors release cytokines that can stimulate the production of megakaryocytes in the marrow and elevate the platelet count. This can result in an indirect increase of VEGF delivery to tumors.

15 VEGF is implicated in several other pathological conditions associated with enhanced angiogenesis. For example, VEGF plays a role in both psoriasis and rheumatoid arthritis. Diabetic retinopathy is associated with high intraocular levels of VEGF. Inhibition of VEGF function may result in infertility by blockade of corpus luteum function. Direct demonstration of the importance of VEGF in tumor growth has 20 been achieved using dominant negative VEGF receptors to block in vivo proliferation, as well as blocking antibodies to VEGF39 or to VEGFr2.

25 The use of small interfering nucleic acid molecules targeting VEGF and corresponding receptors and ligands therefore provides a class of novel therapeutic agents that can be used in the diagnosis of and the treatment of cancer, proliferative diseases, or any other disease or condition that responds to modulation of VEGF and/or VEGFr genes.

Examples:

The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

30 Example 1: Tandem synthesis of siNA constructs

Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example, a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siNA synthesis in support of high 5 throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siNA 10 sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even though only one molecule has a dimethoxytrityl group. Because the strands form a 15 stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example, by using a C18 cartridge.

Standard phosphoramidite synthesis chemistry is used up to the point of introducing a tandem linker, such as an inverted deoxy abasic succinate or glyceryl 20 succinate linker (see **Figure 1**) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexaflurorophosphate (PyBrOP). After the linker is coupled, standard synthesis chemistry is utilized to complete synthesis of the second 25 sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50mM NaOAc or 1.5M NH₄H₂CO₃.

Purification of the siNA duplex can be readily accomplished using solid phase extraction, for example using a Waters C18 SepPak 1g cartridge conditioned with 1 30 column volume (CV) of acetonitrile, 2 CV H₂O, and 2 CV 50mM NaOAc. The sample is loaded and then washed with 1 CV H₂O or 50mM NaOAc. Failure sequences are

eluted with 1 CV 14% ACN (Aqueous with 50mM NaOAc and 50mM NaCl). The column is then washed, for example with 1 CV H₂O followed by on-column detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA) over the column, then adding a second CV of 1% aqueous TFA to the column and 5 allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with H₂O followed by 1 CV 1M NaCl and additional H₂O. The siNA duplex product is then eluted, for example, using 1 CV 20% aqueous CAN.

10 **Figure 2** provides an example of MALDI-TOF mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siNA, and two peaks presumably corresponding to the separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA construct 15 only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

Example 2: Identification of potential siNA target sites in any RNA sequence

20 The sequence of an RNA target of interest, such as a viral or human mRNA transcript, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having complementarity to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for 25 example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siNA molecules targeting those sites. Various parameters can be used to determine which sites are the most suitable target sites within 30 the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the

degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be chosen to screen siNA molecules for efficacy, for example by using *in vitro* RNA cleavage assays, 5 cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

10 Example 3: Selection of siNA molecule target sites in a RNA

The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

1. The target sequence is parsed *in silico* into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target 15 sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.
2. In some instances the siNAs correspond to more than one target sequence; such would be the case for example in targeting different transcripts of the same gene, 20 targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences 25 that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siNA to target specifically the mutant sequence and not effect the expression of the normal sequence.
3. In some instances the siNA subsequences are absent in one or more sequences while 30 present in the desired target sequence; such would be the case if the siNA targets a

gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

5 4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.

10 5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.

15 6. The ranked siNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic and can potentially interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.

20 7. The ranked siNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.

25 8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siNA duplex (see **Tables II and III**). If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced

30 by TT prior to synthesizing the oligos.

9. The siNA molecules are screened in an *in vitro*, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.

In an alternate approach, a pool of siNA constructs specific to a VEGF and/or VEGFr target sequence is used to screen for target sites in cells expressing VEGF and/or VEGFr RNA, such as HUVEC, HMVEC, or A375 cells. The general strategy used in this approach is shown in **Figure 9**. A non-limiting example of such is a pool comprising sequences having any of SEQ ID NOS 1-2455. Cells expressing VEGF and/or VEGFr (e.g., HUVEC, HMVEC, or A375 cells) are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with VEGF and/or VEGFr inhibition are sorted. The pool of siNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example **Figure 7** and **Figure 8**). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased proliferation, decreased VEGF and/or VEGFr mRNA levels or decreased VEGF and/or VEGFr protein expression), are sequenced to determine the most suitable target site(s) within the target VEGF and/or VEGFr RNA sequence.

Example 4: VEGF and/or VEGFr targeted siNA design

siNA target sites were chosen by analyzing sequences of the VEGF and/or VEGFr RNA target and optionally prioritizing the target sites on the basis of folding (structure of any given sequence analyzed to determine siNA accessibility to the target), by using a library of siNA molecules as described in Example 3, or alternately by using an *in vitro* siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are optionally individually analyzed by computer folding to assess whether the siNA molecule can interact with the target sequence. Varying the length of the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA duplexes or varying length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

Chemically modified siNA constructs are designed to provide nuclease stability for systemic administration *in vivo* and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical modifications as described herein are introduced synthetically using synthetic methods 5 described herein and those generally known in the art. The synthetic siNA constructs are then assayed for nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantify RNAi activity. Synthetic siNA constructs that 10 possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example **Figure 11**).

15 Example 5: Chemical Synthesis and Purification of siNA

siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using 20 methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can be synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman *et al.*, US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098; 25 6,362,323; 6,437,117; 6,469,158; Scaringe *et al.*, US Patent Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-dimethoxytrityl, 2'-O-tert-butyldimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphosphoroamidite groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine, 30 N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2'-O-Silyl Ethers can be

used in conjunction with acid-labile 2'-O-orthoester protecting groups in the synthesis of RNA as described by Scaringe *supra*. Differing 2' chemistries can require different protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl protection as described by Usman *et al.*, US Patent 5,631,360, incorporated by reference herein in its entirety).

During solid phase synthesis, each nucleotide is added sequentially (3'- to 5'- direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5'-end of the first nucleoside. The support is then washed and any unreacted 5'-hydroxyl groups are capped with a capping reagent such as acetic anhydride to yield inactive 5'-acetyl moieties. The trivalent phosphorus linkage is then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5'-O-protecting group is cleaved under suitable conditions (e.g., acidic conditions for trityl-based groups and Fluoride for silyl-based groups). The cycle is repeated for each subsequent nucleotide.

Modification of synthesis conditions can be used to optimize coupling efficiency, for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries depending on the particular chemical composition of the siNA to be synthesized. Deprotection and purification of the siNA can be performed as is generally described in Deprotection and purification of the siNA can be performed as is generally described in Usman *et al.*, US 5,831,071, US 6,353,098, US 6,437,117, and Bellon *et al.*, US 6,054,576, US 6,162,909, US 6,303,773, or Scaringe *supra*, incorporated by reference herein in their entireties. Additionally, deprotection conditions can be modified to provide the best possible yield and purity of siNA constructs. For example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides can degrade under inappropriate deprotection conditions. Such oligonucleotides are deprotected using aqueous methylamine at about 35°C for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also comprises ribonucleotides, after deprotection

with aqueous methylamine at about 35°C for 30 minutes, TEA-HF is added and the reaction maintained at about 65°C for an additional 15 minutes.

Example 6: RNAi *in vitro* assay to assess siNA activity

An *in vitro* assay that recapitulates RNAi in a cell-free system is used to evaluate 5 siNA constructs targeting VEGF and/or VEGFr RNA targets. The assay comprises the system described by Tuschl *et al.*, 1999, *Genes and Development*, 13, 3191-3197 and Zamore *et al.*, 2000, *Cell*, 101, 25-33 adapted for use with VEGF and/or VEGFr target RNA. A *Drosophila* extract derived from syncytial blastoderm is used to reconstitute RNAi activity *in vitro*. Target RNA is generated via *in vitro* transcription from an 10 appropriate VEGF and/or VEGFr expressing plasmid using T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 minute at 90°C followed by 1 hour at 37°C, then diluted in lysis buffer (for example 100 mM potassium acetate, 30 15 mM HEPES-KOH at pH 7.4, 2mM magnesium acetate). Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The *Drosophila* lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture 20 containing 50% lysate [vol/vol], RNA (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug.ml creatine phosphokinase, 100 um GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 25 mM. The reactions are pre-assembled on ice and preincubated at 25° C for 10 minutes before adding RNA, then incubated at 25° C for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25 x Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the reaction.

30 Alternately, internally-labeled target RNA for the assay is prepared by *in vitro* transcription in the presence of [α -32P] CTP, passed over a G 50 Sephadex column

by spin chromatography and used as target RNA without further purification. Optionally, target RNA is 5'-³²P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The 5 percentage of cleavage is determined by Phosphor Imager[®] quantitation of bands representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay.

In one embodiment, this assay is used to determine target sites the VEGF and/or VEGFr RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA 10 constructs are screened for RNAi mediated cleavage of the VEGF and/or VEGFr RNA target, for example, by analyzing the assay reaction by electrophoresis of labeled target RNA, or by northern blotting, as well as by other methodology well known in the art.

Example 7: Nucleic acid inhibition of VEGF and/or VEGFr target RNA *in vivo*

15 siNA molecules targeted to the human VEGF and/or VEGFr RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the VEGF and/or VEGFr RNA are given in **Table II and III**.

Two formats are used to test the efficacy of siNAs targeting VEGF and/or VEGFr. 20 First, the reagents are tested in cell culture using, for example, HUVEC, HMVEC, or A375 cells to determine the extent of RNA and protein inhibition. siNA reagents (e.g.; see **Tables II and III**) are selected against the VEGF and/or VEGFr target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, HUVEC, HMVEC, or A375 cells. Relative amounts 25 of target RNA are measured versus actin using real-time PCR monitoring of amplification (eg., ABI 7700 Taqman[®]). A comparison is made to a mixture of oligonucleotide sequences made to unrelated targets or to a randomized siNA control with the same overall length and chemistry, but randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization 30 performed. After an optimal transfection agent concentration is chosen, a RNA time-

course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to determine RNA inhibition.

Delivery of siNA to Cells

Cells (e.g., HUVEC, HMVEC, or A375 cells) are seeded, for example, at 1×10^5 5 cells per well of a six-well dish in EGM-2 (BioWhittaker) the day before transfection. siNA (final concentration, for example 20nM) and cationic lipid (e.g., final concentration 2 μ g/ml) are complexed in EGM basal media (Biowhittaker) at 37°C for 30 minutes in polystyrene tubes. Following vortexing, the complexed siNA is added to each well and incubated for the times indicated. For initial optimization experiments, cells are seeded, 10 for example, at 1×10^3 in 96 well plates and siNA complex added as described. Efficiency of delivery of siNA to cells is determined using a fluorescent siNA complexed with lipid. Cells in 6-well dishes are incubated with siNA for 24 hours, rinsed with PBS and fixed in 2% paraformaldehyde for 15 minutes at room temperature. Uptake of siNA is visualized using a fluorescent microscope.

15 Taqman and Lightcycler quantification of mRNA

Total RNA is prepared from cells following siNA delivery, for example, using Qiagen RNA purification kits for 6-well or Rneasy extraction kits for 96-well assays. For Taqman analysis, dual-labeled probes are synthesized with the reporter dye, FAM or JOE, covalently linked at the 5'-end and the quencher dye TAMRA conjugated to the 3'- 20 end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence Detector using 50 μ l reactions consisting of 10 μ l total RNA, 100 nM forward primer, 900 nM reverse primer, 100 nM probe, 1X TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5 mM MgCl₂, 300 μ M each dATP, dCTP, dGTP, and dTTP, 10U RNase Inhibitor (Promega), 1.25U AmpliTaq Gold (PE-Applied Biosystems) and 25 10U M-MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of 30 minutes at 48°C, 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Quantitation of mRNA levels is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 33, 11 ng/rxn) and normalizing to β -actin or GAPDH mRNA in parallel TaqMan reactions. For each 30 gene of interest an upper and lower primer and a fluorescently labeled probe are

designed. Real time incorporation of SYBR Green I dye into a specific PCR product can be measured in glass capillary tubes using a lightcyler. A standard curve is generated for each primer pair using control cRNA. Values are represented as relative expression to GAPDH in each sample.

5 Western blotting

Nuclear extracts can be prepared using a standard micro preparation technique (see for example Andrews and Faller, 1991, *Nucleic Acids Research*, 19, 2499). Protein extracts from supernatants are prepared, for example using TCA precipitation. An equal volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and 10 pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris-Glycine (supernatant extracts) polyacrylamide gel and transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody 15 for 16 hour at 4°C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

Example 8: Animal Models useful to evaluate the down-regulation of VEGF and/or VEGFr gene expression

20 There are several animal models in which the anti-angiogenesis effect of nucleic acids of the present invention, such as siRNA, directed against VEGF, VEGFr1, VEGFr2 and/or VEGFr3 mRNAs can be tested. Typically a corneal model has been used to study angiogenesis in rat and rabbit since recruitment of vessels can easily be followed in this normally avascular tissue (Pandey *et al.*, 1995 *Science* 268: 567-569). In these 25 models, a small Teflon or Hydron disk pretreated with an angiogenesis factor (e.g. bFGF or VEGF) is inserted into a pocket surgically created in the cornea. Angiogenesis is monitored 3 to 5 days later. siRNA directed against VEGF, VEGFr1, VEGFr2 and/or VEGFr3 mRNAs are delivered in the disk as well, or dropwise to the eye over the time course of the experiment. In another eye model, hypoxia has been shown to cause both 30 increased expression of VEGF and neovascularization in the retina (Pierce *et al.*, 1995

Proc. Natl. Acad. Sci. USA. 92: 905-909; Shweiki *et al.*, 1992 *J. Clin. Invest.* 91: 2235-2243).

In human glioblastomas, it has been shown that VEGF is at least partially responsible for tumor angiogenesis (Plate *et al.*, 1992 *Nature* 359, 845). Animal models 5 have been developed in which glioblastoma cells are implanted subcutaneously into nude mice and the progress of tumor growth and angiogenesis is studied (Kim *et al.*, 1993 *supra*; Millauer *et al.*, 1994 *supra*).

Another animal model that addresses neovascularization involves Matrigel, an extract of basement membrane that becomes a solid gel when injected subcutaneously 10 (Passaniti *et al.*, 1992 *Lab. Invest.* 67: 519-528). When the Matrigel is supplemented with angiogenesis factors such as VEGF, vessels grow into the Matrigel over a period of 3 to 5 days and angiogenesis can be assessed. Again, nucleic acids directed against VEGFr mRNAs are delivered in the Matrigel.

Several animal models exist for screening of anti-angiogenic agents. These 15 include corneal vessel formation following corneal injury (Burger *et al.*, 1985 *Cornea* 4: 35-41; Lepri, *et al.*, 1994 *J. Ocular Pharmacol.* 10: 273-280; Ormerod *et al.*, 1990 *Am. J. Pathol.* 137: 1243-1252) or intracorneal growth factor implant (Grant *et al.*, 1993 *Diabetologia* 36: 282-291; Pandey *et al.* 1995 *supra*; Zieche *et al.*, 1992 *Lab. Invest.* 67: 711-715), vessel growth into Matrigel matrix containing growth factors (Passaniti *et al.*, 1992 *supra*), female reproductive organ neovascularization following hormonal manipulation (Shweiki *et al.*, 1993 *Clin. Invest.* 91: 2235-2243), several models involving inhibition of tumor growth in highly vascularized solid tumors (O'Reilly *et al.*, 1994 *Cell* 79: 315-328; Senger *et al.*, 1993 *Cancer and Metas. Rev.* 12: 303-324; Takahasi *et al.*, 1994 *Cancer Res.* 54: 4233-4237; Kim *et al.*, 1993 *supra*), and transient 20 25 hypoxia-induced neovascularization in the mouse retina (Pierce *et al.*, 1995 *Proc. Natl. Acad. Sci. USA.* 92: 905-909). Other model systems to study tumor angiogenesis are reviewed by Folkman, 1985 *Adv. Cancer. Res.* 43, 175.

Ocular Models of Angiogenesis

The cornea model, described in Pandey *et al. supra*, is the most common and well 30 characterized model for screening anti-angiogenic agent efficacy. This model involves

an avascular tissue into which vessels are recruited by a stimulating agent (growth factor, thermal or alkalai burn, endotoxin). The corneal model utilizes the intrastromal corneal implantation of a Teflon pellet soaked in a VEGF-Hydrone solution to recruit blood vessels toward the pellet, which can be quantitated using standard microscopic and 5 image analysis techniques. To evaluate their anti-angiogenic efficacy, nucleic acids are applied topically to the eye or bound within Hydrone on the Teflon pellet itself. This avascular cornea as well as the Matrigel (see below) provide for low background assays. While the corneal model has been performed extensively in the rabbit, studies in the rat have also been conducted.

10 The mouse model (Passaniti et al., *supra*) is a non-tissue model that utilizes Matrigel, an extract of basement membrane (Kleinman et al., 1986) or Millipore® filter disk, which can be impregnated with growth factors and anti-angiogenic agents in a liquid form prior to injection. Upon subcutaneous administration at body temperature, the Matrigel or Millipore® filter disk forms a solid implant. VEGF embedded in the 15 Matrigel or Millipore® filter disk is used to recruit vessels within the matrix of the Matrigel or Millipore® filter disk which can be processed histologically for endothelial cell specific vWF (factor VIII antigen) immunohistochemistry, Trichrome-Masson stain, or hemoglobin content. Like the cornea, the Matrigel or Millipore® filter disk is avascular; however, it is not tissue. In the Matrigel or Millipore® filter disk model, 20 nucleic acids are administered within the matrix of the Matrigel or Millipore® filter disk to test their anti-angiogenic efficacy. Thus, delivery issues in this model, as with delivery of nucleic acids by Hydrone- coated Teflon pellets in the rat cornea model, may be less problematic due to the homogeneous presence of the nucleic acid within the respective matrix.

25 Additionally, siNA molecules of the invention targeting VEGF and/or VEGFr (e.g. VEGFR1, VEGFR2, and/or VEGFR3) can be assessed for activity transgenic mice to determine whether modulation of VEGF and/or VEGFr can inhibit optic neovascularization. Animal models of choroidal neovascularization are described in, for example, Mori et al., 2001, *Journal of Cellular Physiology*, 188, 253; Mori et al., 2001, 30 *American Journal of Pathology*, 159, 313; Ohno-Matsui et al., 2002, *American Journal of Pathology*, 160, 711; and Kwak et al., 2000, *Investigative Ophthalmology & Visual*

Science, 41, 3158. VEGF plays a central role in causing retinal neovascularization. Increased expression of VEGFR2 in retinal photoreceptors of transgenic mice stimulates neovascularization within the retina, and a blockade of VEGFR2 signaling has been shown to inhibit retinal choroidal neovascularization (CNV) (Mori *et al.*, 2001, *J. Cell. Physiol.*, 188, 253).

CNV is laser induced in, for example, adult C57BL/6 mice. The mice are also given an intravitreous, periocular or a subretinal injection of VEGF and/or VEGFr (e.g., VEGFR2) siNA in each eye. Intravitreous injections are made using a Harvard pump microinjection apparatus and pulled glass micropipets. Then a micropipette is passed 10 through the sclera just behind the limbus into the vitreous cavity. The subretinal injections are made using a condensing lens system on a dissecting microscope. The pipet tip is then passed through the sclera posterior to the limbus and positioned above the retina. Five days after the injection of the vector the mice are anesthetized with ketamine hydrochloride (100 mg/kg body weight), 1% tropicamide is also used to dilate 15 the pupil, and a diode laser photocoagulation is used to rupture Bruch's membrane at three locations in each eye. A slit lamp delivery system and a hand-held cover slide are used for laser photocoagulation. Burns are made in the 9, 12, and 3 o'clock positions 2-3 disc diameters from the optic nerve (Mori *et al.*, *supra*).

The mice typically develop subretinal neovascularization due to the expression of 20 VEGF in photoreceptors beginning at prenatal day 7. At prenatal day 21, the mice are anesthetized and perfused with 1 ml of phosphate-buffered saline containing 50 mg/ml of fluorescein-labeled dextran. Then the eyes are removed and placed for 1 hour in a 10% phosphate-buffered formalin. The retinas are removed and examined by fluorescence microscopy (Mori *et al.*, *supra*).

25 Fourteen days after the laser induced rupture of Bruch's membrane, the eyes that received intravitreous and subretinal injection of siNA are evaluated for smaller appearing areas of CNV, while control eyes are evaluated for large areas of CNV. The eyes that receive intravitreous injections or a subretinal injection of siNA are also evaluated for fewer areas of neovascularization on the outer surface of the retina and 30 potential abortive sprouts from deep retinal capillaries that do not reach the retinal surface compared to eyes that did not receive an injection of siNA.

*Tumor Models of Angiogenesis**Use of murine models*

For a typical systemic study involving 10 mice (20 g each) per dose group, 5 doses (1, 3, 10, 30 and 100 mg/kg daily over 14 days continuous administration), 5 approximately 400 mg of siRNA, formulated in saline is used. A similar study in young adult rats (200 g) requires over 4 g. Parallel pharmacokinetic studies involve the use of similar quantities of siRNA further justifying the use of murine models.

Lewis lung carcinoma and B-16 melanoma murine models

Identifying a common animal model for systemic efficacy testing of nucleic acids is 10 an efficient way of screening siRNA for systemic efficacy.

The Lewis lung carcinoma and B-16 murine melanoma models are well accepted models of primary and metastatic cancer and are used for initial screening of anti-cancer agents. These murine models are not dependent upon the use of immunodeficient mice, are relatively inexpensive, and minimize housing concerns. Both the Lewis lung and B- 15 16 melanoma models involve subcutaneous implantation of approximately 10^6 tumor cells from metastatically aggressive tumor cell lines (Lewis lung lines 3LL or D122, LLc-LN7; B-16-BL6 melanoma) in C57BL/6J mice. Alternatively, the Lewis lung model can be produced by the surgical implantation of tumor spheres (approximately 0.8 mm in diameter). Metastasis also can be modeled by injecting the tumor cells directly 20 intravenously. In the Lewis lung model, microscopic metastases can be observed approximately 14 days following implantation with quantifiable macroscopic metastatic tumors developing within 21-25 days. The B-16 melanoma exhibits a similar time course with tumor neovascularization beginning 4 days following implantation. Since both primary and metastatic tumors exist in these models after 21-25 days in the same animal, 25 multiple measurements can be taken as indices of efficacy. Primary tumor volume and growth latency as well as the number of micro- and macroscopic metastatic lung foci or number of animals exhibiting metastases can be quantitated. The percent increase in lifespan can also be measured. Thus, these models provide suitable primary efficacy assays for screening systemically administered siRNA nucleic acids and siRNA nucleic 30 acid formulations.

In the Lewis lung and B-16 melanoma models, systemic pharmacotherapy with a wide variety of agents usually begins 1-7 days following tumor implantation/inoculation with either continuous or multiple administration regimens. Concurrent pharmacokinetic studies can be performed to determine whether sufficient tissue levels of siRNA can be 5 achieved for pharmacodynamic effect to be expected. Furthermore, primary tumors and secondary lung metastases can be removed and subjected to a variety of *in vitro* studies (*i.e.* target RNA reduction).

In addition, animal models are useful in screening compounds, eg. siRNA molecules, for efficacy in treating renal failure, such as a result of autosomal dominant 10 polycystic kidney disease (ADPKD). The Han:SPRD rat model, mice with a targeted mutation in the Pkd2 gene and congenital polycystic kidney (cpk) mice, closely resemble human ADPKD and provide animal models to evaluate the therapeutic effect of siRNA constructs that have the potential to interfere with one or more of the pathogenic elements of ADPKD mediated renal failure, such as angiogenesis. Angiogenesis may be 15 necessary in the progression of ADPKD for growth of cyst cells as well as increased vascular permeability promoting fluid secretion into cysts. Proliferation of cystic epithelium is also a feature of ADPKD because cyst cells in culture produce soluble vascular endothelial growth factor (VEGF). VEGFr1 has also been detected in epithelial cells of cystic tubules but not in endothelial cells in the vasculature of cystic kidneys or 20 normal kidneys. VEGFr2 expression is increased in endothelial cells of cyst vessels and in endothelial cells during renal ischemia-reperfusion. It is proposed that inhibition of VEGF receptors with anti-VEGFr1 and anti-VEGFr2 siRNA molecules would attenuate cyst formation, renal failure and mortality in ADPKD. Anti-VEGFr2 siRNA molecules would therefore be designed to inhibit angiogenesis involved in cyst formation. As 25 VEGFr1 is present in cystic epithelium and not in vascular endothelium of cysts, it is proposed that anti-VEGFr1 siRNA molecules would attenuate cystic epithelial cell proliferation and apoptosis which would in turn lead to less cyst formation. Further, it is proposed that VEGF produced by cystic epithelial cells is one of the stimuli for angiogenesis as well as epithelial cell proliferation and apoptosis. The use of Han:SPRD 30 rats (see for example Kaspareit-Rittinghausen *et al.*, 1991, *Am.J.Pathol.* 139, 693-696), mice with a targeted mutation in the Pkd2 gene (Pkd2^{-/-} mice, see for example Wu *et al.*, 2000, *Nat.Genet.* 24, 75-78) and cpk mice (see for example Woo *et al.*, 1994, *Nature*,

368, 750-753) all provide animal models to study the efficacy of siRNA molecules of the invention against VEGFr1 and VEGFr2 mediated renal failure.

VEGF, VEGFr1 VGFR2 and/or VEGFr3 protein levels can be measured clinically or experimentally by FACS analysis. VEGF, VEGFr1 VGFR2 and/or VEGFr3 encoded 5 mRNA levels are assessed by Northern analysis, RNase-protection, primer extension analysis and/or quantitative RT-PCR. siRNA nucleic acids that block VEGF, VEGFr1 VGFR2 and/or VEGFr3 protein encoding mRNAs and therefore result in decreased levels of VEGF, VEGFr1 VGFR2 and/or VEGFr3 activity by more than 20% *in vitro* can be identified.

10 Example 9: RNAi mediated inhibition of VEGFr1 RNA expression

siNA constructs (**Table III**) are tested for efficacy in reducing VEGF and/or VEGFr RNA expression in, for example, HUVEC, HMVEC, or A375 cells. Cells are plated approximately 24 hours before transfection in 96-well plates at 5,000-7,500 cells/well, 100 μ l/well, such that at the time of transfection cells are 70-90% confluent. 15 For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50 μ l/well and incubated for 20 min. at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150 μ l. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24h in the 20 continued presence of the siNA transfection mixture. At 24h, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The 25 triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is determined.

Figure 13 shows a non-limiting example of reduction of VEGFr1 mRNA in A375 cells mediated by chemically-modified siNAs that target VEGFr1 mRNA. A549 cells 30 were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. A screen of siNA constructs (Stabilization “Stab” chemistries are shown in **Table IV**, constructs are

referred to by RPI number, see **Table III**) comprising Stab 4/5 chemistry (Sirna/RPI 31190/31193), Stab 1/2 chemistry (Sirna/RPI 31183/31186 and Sirna/RPI 31184/31187), and unmodified RNA (Sirna/RPI 30075/30076) were compared to untreated cells, matched chemistry inverted control siNA constructs (Sirna/RPI 31208/31211, Sirna/RPI 5 31201/31204, Sirna/RPI 31202/31205, and Sirna/RPI 30077/30078), scrambled siNA control constructs (Scram1 and Scram2), and cells transfected with lipid alone (transfection control). As shown in the figure, all of the siNA constructs significantly reduce VEGFr1 RNA expression. Additional stabilization chemistries as described in **Table IV** are similarly assayed for activity. These siNA constructs are compared to 10 appropriate matched chemistry inverted controls. In addition, the siNA constructs are also compared to untreated cells, cells transfected with lipid and scrambled siNA constructs, and cells transfected with lipid alone (transfection control).

Example 10: siNA-mediated inhibition of angiogenesis *in vivo*

Evaluation of siNA molecules in the rat cornea model of VEGF induced angiogenesis

15 The purpose of this study was to assess the anti-angiogenic activity of siNA targeted against VEGFR1, using the rat cornea model of VEGF induced angiogenesis. The siNA molecules referred to in **Figure 12** have matched inverted controls which are inactive since they are not able to interact with the RNA target. The siNA molecules and VEGF were co-delivered using the filter disk method. Nitrocellulose filter disks 20 (Millipore[®]) of 0.057 diameter were immersed in appropriate solutions and were surgically implanted in rat cornea as described by Pandey *et al.*, *supra*.

The stimulus for angiogenesis in this study was the treatment of the filter disk with 30 μ M VEGF, which is implanted within the cornea's stroma. This dose yields reproducible neovascularization stemming from the pericorneal vascular plexus growing 25 toward the disk in a dose-response study 5 days following implant. Filter disks treated only with the vehicle for VEGF show no angiogenic response. The siNA were co-administered with VEGF on a disk in three different siNA concentrations. One concern with the simultaneous administration is that the siNA would not be able to inhibit angiogenesis since VEGF receptors can be stimulated. However, Applicant has observed 30 that in low VEGF doses, the neovascular response reverts to normal suggesting that the VEGF stimulus is essential for maintaining the angiogenic response. Blocking the

production of VEGF receptors using simultaneous administration of anti-VEGF-R mRNA siNA could attenuate the normal neovascularization induced by the filter disk treated with VEGF.

Materials and Methods:

5 *Test Compounds and Controls*

R&D Systems VEGF, carrier free at 75 µM in 82 mM Tris-Cl, pH 6.9
siNA, 1.67 µG/µL, SITE 2340 (SIRNA/RPI 29695/29699) sense/antisense
10 siNA, 1.67 µG/µL, INVERTED CONTROL FOR SITE 2340 (SIRNA/RPI
29983/29984) sense/antisense
 siNA 1.67 µg/µL, Site 2340 (Sirma/RPI 30196/30416) sense/antisense

Animals

15 Harlan Sprague-Dawley Rats, Approximately 225-250g
 45 males, 5 animals per group.

Husbandry

20 Animals are housed in groups of two. Feed, water, temperature and humidity are determined according to Pharmacology Testing Facility performance standards (SOP's) which are in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (NRC). Animals are acclimated to the facility for at least 7 days prior to experimentation. During this time, animals are observed for overall health and sentinels are bled for baseline serology.

Experimental Groups

25 Each solution (VEGF and siNAs) was prepared as a 1X solution for final concentrations shown in the experimental groups described in **Table III**.

siNA Annealing Conditions

siNA sense and antisense strands are annealed for 1 minute in H₂O at 1.67mg/mL/strand followed by a 1 hour incubation at 37°C producing 3.34 mg/mL of duplexed siNA. For the 20 μ g/eye treatment, 6 μ Ls of the 3.34 mg/mL duplex is injected into the eye (see below). The 3.34 mg/mL duplex siNA can then be serially diluted for 5 dose response assays.

Preparation of VEGF Filter Disk

For corneal implantation, 0.57 mm diameter nitrocellulose disks, prepared from 10 0.45 μ m pore diameter nitrocellulose filter membranes (Millipore Corporation), were soaked for 30 min in 1 μ L of 75 μ M VEGF in 82 mM Tris-HCl (pH 6.9) in covered petri dishes on ice. Filter disks soaked only with the vehicle for VEGF (83 mM Tris-Cl pH 6.9) elicit no angiogenic response.

Corneal surgery

15

The rat corneal model used in this study was a modified from Koch *et al. Supra* and Pandey *et al., supra*. Briefly, corneas were irrigated with 0.5% povidone iodine solution followed by normal saline and two drops of 2% lidocaine. Under a dissecting microscope (Leica MZ-6), a stromal pocket was created and a presoaked filter disk (see 20 above) was inserted into the pocket such that its edge was 1 mm from the corneal limbus.

Intraconjunctival injection of test solutions

Immediately after disk insertion, the tip of a 40-50 μ m OD injector (constructed in 25 our laboratory) was inserted within the conjunctival tissue 1 mm away from the edge of the corneal limbus that was directly adjacent to the VEGF-soaked filter disk. Six hundred nanoliters of test solution (siNA, inverted control or sterile water vehicle) were dispensed at a rate of 1.2 μ L/min using a syringe pump (KD Scientific). The injector was then removed, serially rinsed in 70% ethanol and sterile water and immersed in sterile water between each injection. Once the test solution was injected, closure of the eyelid was 30 maintained using microaneurism clips until the animal began to recover gross motor activity. Following treatment, animals were warmed on a heating pad at 37°C.

Quantitation of angiogenic response

Five days after disk implantation, animals were euthanized following administration of 0.4 mg/kg atropine and corneas were digitally imaged. The neovascular surface area (NSA, expressed in pixels) was measured *postmortem* from blood-filled corneal vessels using computerized morphometry (Image Pro Plus, Media Cybernetics, v2.0). The individual mean NSA was determined in triplicate from three regions of identical size in the area of maximal neovascularization between the filter disk and the limbus. The number of pixels corresponding to the blood-filled corneal vessels in these regions was summated to produce an index of NSA. A group mean NSA was then calculated. Data from each treatment group were normalized to VEGF/siNA vehicle-treated control NSA and finally expressed as percent inhibition of VEGF-induced angiogenesis.

Statistics

15

After determining the normality of treatment group means, group mean percent inhibition of VEGF-induced angiogenesis was subjected to a one-way analysis of variance. This was followed by two post-hoc tests for significance including Dunnett's (comparison to VEGF control) and Tukey-Kramer (all other group mean comparisons) at 20 alpha = 0.05. Statistical analyses were performed using JMP v.3.1.6 (SAS Institute).

Results of the study are graphically represented in **Figures 12 and 16**. As shown in **Figure 12**, VEGFr1 site 4229 active siNA (Sirna/RPI 29695/29699) at three concentrations was effective at inhibiting angiogenesis compared to the inverted siNA control (Sirna/RPI 29983/29984) and the VEGF control. A chemically modified version 25 of the VEGFr1 site 4229 active siNA comprising a sense strand having 2'-deoxy-2'-fluoro pyrimidines and ribo purines with 5' and 3' terminal inverted deoxyabasic residues and an antisense strand having 2'-deoxy-2'-fluoro pyrimidines and ribo purines with a terminal 3'-phosphorothioate internucleotide linkage (Sirna/RPI 30196/30416), showed similar inhibition. Furthermore, VEGFr1 site 349 active siNA 30 having "Stab 9/10" chemistry (Compound No. 31270/31273) was tested for inhibition of VEGF-induced angiogenesis at three different concentrations (2.0 ug, 1.0 ug, and 0.1 ug dose response) as compared to a matched chemistry inverted control siNA construct

(Compound No. 31276/31279) at each concentration and a VEGF control in which no siNA was administered. As shown in **Figure 16**, the active siNA construct having “Stab 9/10” chemistry (Compound No. 31270/31273) is highly effective in inhibiting VEGF-induced angiogenesis in the rat corneal model compared to the matched chemistry 5 inverted control siNA at concentrations from 0.1 ug to 2.0 ug. These results demonstrate that siNA molecules having different chemically modified compositions, such as the modifications described herein, are capable of significantly inhibiting angiogenesis *in vivo*.

Evaluation of siNA molecules in the mouse coroidal model of neovascularization.

10 Intraocular Administration of siNA

Female C57BL/6 mice (4-5 weeks old) were anesthetized with a 0.2 ml of a mixture of ketamine/xylazine (8:1), and the pupils were dilated with a single drop of 1% tropicamide. Then a 532nm diode laser photocoagulation (75 μ m spot size, 0.1-second duration, 120 mW) was used to generate three laser spots in each eye surrounding the 15 optic nerve by using a hand-held coverslip as a contact lens. A bubble formed at the laser spot indicating a rupture of the Bruch's membrane. Next, the laser spots were evaluated for the presence of CNV on day 17 after laser treatment.

After laser induction of multiple CNV lesions in mice, the siNA was administered by intraocular injections under a dissecting microscope. Intravitreous 20 injections were performed with a Harvard pump microinjection apparatus and pulled glass micropipets. Each micropipet was calibrated to deliver 1 μ L of vehicle containing 0.5 ug or 1.5 ug of siNA, inverted control siNA, or saline. The mice were anesthetized, pupils were dilated, and, the sharpened tip of the micropipet was passed through the sclera, just behind the limbus into the vitreous cavity, and the foot switch was depressed. 25 The injection was repeated at day 7 after laser photocoagulation.

At the time of death, mice were anesthetized (ketamine/xylazine mixture, 8:1) and perfused through the heart with 1 ml PBS containing 50 mg/ml fluorescein-labeled dextran (FITC-Dextran, 2 million average molecular weight, Sigma). The eyes were removed and fixed for overnight in 1% phosphate-buffered 4% Formalin. The cornea 30 and the lens were removed and the neurosensory retina was carefully dissected from the

eyecup. Five radial cuts were made from the edge of the eyecup to the equator; the sclera-choroid-retinal pigment epithelium (RPE) complex was flat-mounted, with the sclera facing down, on a glass slide in Aquamount. Flat mounts were examined with a Nikon fluorescence microscope. A laser spot with green vessels was scored CNV-positive, and a laser spot lacking green vessels was scored CNV-negative. Flatmounts were examined by fluorescence microscopy (Axioskop; Carl Zeiss, Thornwood, NY), and images were digitized with a three-color charge-coupled device (CCD) video camera and a frame grabber. Image-analysis software (Image-Pro Plus; Media Cybernetics, Silver Spring, MD) was used to measure the total area of hyperfluorescence associated with each burn, corresponding to the total fibrovascular scar. The areas within each eye were averaged to give one experimental value per eye for plotting the areas.

Measurement of VEGFr1 expression was also determined using RT-PCR and/or real-time PCR. Retinal RNA was isolated by a Rnaeasy kit, and reverse transcription was performed with approximately 0.5 μ g total RNA, reverse transcriptase (SuperScript II), and 5.0 μ M oligo-d(T) primer. PCR amplification was performed using primers specific for VEGFR-1 (5'- AAGATGCCAGCCGAAGGAGA-3', SEQ ID NO: 2456) and (5'-GGCTCGGCACCTATAGACA-3', SEQ ID NO: 2457). Titrations were determined to ensure that PCR reactions were performed in the linear range of amplification. Mouse S16 ribosomal protein primers (5'-CACTGCAAACGGGGAAATGG-3', SEQ ID NO: 2458 and 5'-TGAGATGGACTGTCGGATGG-3', SEQ ID NO: 2459) were used to provide an internal control for the amount of template in the PCR reactions.

VEGFr1 site 349 active siNA having “Stab 9/10” chemistry (Compound No. 31270/31273, Table III) was tested for inhibition of VEGF-induced neovascularization at two different concentrations (1.5 μ g, and 0.5 μ g dose response) as compared to a matched chemistry 1.5 μ g inverted control siNA construct (Compound No. 31276/31279, Table III) and a saline control. As shown in **Figure 17**, the active siNA construct having “Stab 9/10” chemistry is highly effective in inhibiting VEGFr1 induced neovascularization (57% inhibition) in the C57BL/6 mice intraocular delivery model compared to the matched chemistry inverted control siNA. The active siNA construct was also highly effective in inhibiting VEGFr1 induced neovascularization (66% inhibition) compared to the saline control. Additionally, RT-PCR analysis of VEGFr1 site 349 siNA having “Stab 9/10” chemistry (Compound No. 31270/31273, Table III)

showed significant reduction in the level of VEGFr1 mRNA compared to the inverted siNA construct (Compound No. 31276/31279, Table III) and saline. Furthermore, ELISA analysis of VEGFr1 protein using the active siNA and inverted control siNA above showed significant reduction in the level of VEGFr1 protein expression using the 5 active siNA compared to the inactive siNA construct. These results demonstrate that siNA molecules having different chemically modified compositions, such as the modifications described herein, are capable of significantly inhibiting neovascularization as shown in this model of intraocular administration.

Periocular Administration of siNA

10 Female C57BL/6 mice (4-5 weeks old) were anesthetized with a 0.2 ml of a mixture of ketamine/xylazine (8:1), and the pupils were dilated with a single drop of 1% tropicamide. Then a 532nm diode laser photocoagulation (75 μ m spot size, 0.1-s duration, 120 mW) was used to generate three laser spots in each eye surrounding the optic nerve by using a hand-held coverslip as a contact lens. A bubble formed at the 15 laser spot indicating a rupture of the Bruch's membrane. Next, the laser spots were evaluated for the presence of CNV on day 17 after laser treatment.

20 After laser induction of multiple CNV lesions in mice, the siNA was administered via periocular injections under a dissecting microscope. Periocular injections were performed with a Harvard pump microinjection apparatus and pulled glass micropipets. Each micropipet was calibrated to deliver 5 μ L of vehicle containing test siNA at concentrations of 0.5 ug or 1.5 ug of siNA. The mice were anesthetized, pupils were dilated, and, the sharpened tip of the micropipet was passed, and the foot switch was depressed. Periocular injections were given daily starting at day 1 through day 14 after laser photocoagulation.

25 At the time of death, mice were anesthetized (ketamine/xylazine mixture, 8:1) and perfused through the heart with 1 mL PBS containing 50 mg/mL fluorescein-labeled dextran (FITC-Dextran, 2 million average molecular weight, Sigma). The eyes were removed and fixed overnight in 1% phosphate-buffered 4% Formalin. The cornea and the lens were removed and the neurosensory retina was carefully dissected from the 30 eyecup. Five radial cuts were made from the edge of the eyecup to the equator; the sclera-choroid-retinal pigment epithelium (RPE) complex was flat-mounted, with the

sclera facing down, on a glass slide in Aquamount. Flat mounts were examined with a Nikon fluorescence microscope. A laser spot with green vessels was scored CNV-positive, and a laser spot lacking green vessels was scored CNV-negative. Flatmounts were examined by fluorescence microscopy (Axioskop; Carl Zeiss, Thornwood, NY) and 5 images were digitized with a three-color charge-coupled device (CCD) video camera and a frame grabber. Image-analysis software (Image-Pro Plus; Media Cybernetics, Silver Spring, MD) was used to measure the total area of hyperfluorescence associated with each burn, corresponding to the total fibrovascular scar. The areas within each eye were averaged to give one experimental value per eye.

10 VEGFr1 site 349 active siNA having “Stab 9/10” chemistry (Compound No. 31270/31273, Table III) was tested for inhibition of VEGF-induced neovascularization at two different concentrations (1.5 ug, and 0.5 ug dose response) as compared to a matched chemistry saline control and 0.5 ug inverted control siRNA construct (Compound No. 31276/31279, Table III). As shown in **Figure 18**, the active siNA 15 construct having “Stab 9/10” chemistry (Compound No. 31270/31273) is effective in inhibiting VEGFr1 induced neovascularization (20% inhibition) in the C57BL/6 mice periocular delivery model compared to the matched chemistry inverted control siNA. The active siNA construct was also highly effective in inhibiting VEGFr1 induced neovascularization (54% inhibition) compared to the saline control. In an additional 20 assay shown in **Figure 19**, VEGFr1 site 349 active siNA having “Stab 9/10” chemistry (Compound No. 31270/31273) at two concentrations was effective at inhibiting neovascularization in CNV lesions compared to the inverted siNA control and the saline control. As shown in **Figure 19**, the active siNA construct having “Stab 9/10” chemistry 25 (Compound No. 31270/31273) is effective in inhibiting VEGFr1 induced neovascularization (43% inhibition) in the C57BL/6 mice periocular delivery model compared to the matched chemistry inverted control siNA. The active siNA construct was also effective in inhibiting VEGFr1 induced neovascularization (33% inhibition) compared to the saline control. These results demonstrate that siNA molecules having different chemically modified compositions, such as the modifications described herein, 30 are capable of significantly inhibiting neovascularization as shown in this model of periocular administration.

Example 11: Indications

The present body of knowledge in VEGF and/or VEGFr research indicates the need for methods to assay VEGF and/or VEGFr activity and for compounds that can regulate VEGF and/or VEGFr expression for research, diagnostic, and therapeutic use. As described herein, the nucleic acid molecules of the present invention can be used in assays to diagnose disease state related of VEGF and/or VEGFr levels. In addition, the nucleic acid molecules can be used to treat disease state related to VEGF and/or VEGFr levels.

Particular conditions and disease states that can be associated with VEGF and/or VEGFr expression modulation include, but are not limited to:

- 10 1) Tumor angiogenesis: Angiogenesis has been shown to be necessary for tumors to grow into pathological size (Folkman, 1971, *PNAS* 76, 5217-5221; Wellstein & Czubayko, 1996, *Breast Cancer Res and Treatment* 38, 109-119). In addition, it allows tumor cells to travel through the circulatory system during metastasis. Increased levels of gene expression of a number of angiogenic factors such as vascular endothelial growth factor (VEGF) have been reported in vascularized and edema-associated brain tumors (Berkman *et al.*, 1993 *J. Clin. Invest.* 91, 153). A more direct demonstration of the role of VEGF in tumor angiogenesis was demonstrated by Jim Kim *et al.*, 1993 *Nature* 362,841 wherein, monoclonal antibodies against VEGF were successfully used to inhibit the growth of rhabdomyosarcoma, glioblastoma multiforme cells in nude mice.
- 15 20 Similarly, expression of a dominant negative mutated form of the flt-1 VEGF receptor inhibits vascularization induced by human glioblastoma cells in nude mice (Millauer *et al.*, 1994, *Nature* 367, 576). Specific tumor/cancer types that can be targeted using the nucleic acid molecules of the invention include but are not limited to the tumor/cancer types described herein.
- 25 30 2) Ocular diseases: Neovascularization has been shown to cause or exacerbate ocular diseases including, but not limited to, macular degeneration, neovascular glaucoma, diabetic retinopathy, myopic degeneration, and trachoma (Norrby, 1997, *APMIS* 105, 417-437). Aiello *et al.*, 1994 *New Engl. J. Med.* 331, 1480, showed that the ocular fluid of a majority of patients suffering from diabetic retinopathy and other retinal disorders contains a high concentration of VEGF. Miller *et al.*, 1994 *Am. J. Pathol.* 145, 574, reported elevated levels of VEGF mRNA in patients suffering from retinal

ischemia. These observations support a direct role for VEGF in ocular diseases. Other factors, including those that stimulate VEGF synthesis, may also contribute to these indications.

3) Dermatological Disorders: Many indications have been identified which may 5 be angiogenesis dependent, including but not limited to, psoriasis, verruca vulgaris, angiofibroma of tuberous sclerosis, pot-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber syndrome, and Osler-Weber-Rendu syndrome (Norrby, *supra*). Intradermal injection of the angiogenic factor b-FGF demonstrated angiogenesis in nude mice (Weckbecker *et al.*, 1992, *Angiogenesis: Key principles-Science-Technology-Medicine*, ed R. Steiner). Detmar *et al.*, 1994 *J. Exp. Med.* 180, 1141 reported that 10 VEGF and its receptors were over-expressed in psoriatic skin and psoriatic dermal microvessels, suggesting that VEGF plays a significant role in psoriasis.

4) Rheumatoid arthritis: Immunohistochemistry and *in situ* hybridization studies 15 on tissues from the joints of patients suffering from rheumatoid arthritis show an increased level of VEGF and its receptors (Fava *et al.*, 1994 *J. Exp. Med.* 180, 341). Additionally, Koch *et al.*, 1994 *J. Immunol.* 152, 4149, found that VEGF-specific 20 antibodies were able to significantly reduce the mitogenic activity of synovial tissues from patients suffering from rheumatoid arthritis. These observations support a direct role for VEGF in rheumatoid arthritis. Other angiogenic factors including those of the present invention may also be involved in arthritis.

5) Endometriosis: Various studies indicate that VEGF is directly implicated in 25 endometriosis. In one study, VEGF concentrations measured by ELISA in peritoneal fluid were found to be significantly higher in women with endometriosis than in women without endometriosis (24.1 ± 15 ng/ml vs 13.3 ± 7.2 ng/ml in normals). In patients with endometriosis, higher concentrations of VEGF were detected in the proliferative phase of the menstrual cycle (33 ± 13 ng/ml) compared to the secretory phase (10.7 ± 5 ng/ml). The cyclic variation was not noted in fluid from normal patients (McLaren *et al.*, 1996, *Human Reprod.* 11, 220-223). In another study, women with moderate to severe 30 endometriosis had significantly higher concentrations of peritoneal fluid VEGF than women without endometriosis. There was a positive correlation between the severity of endometriosis and the concentration of VEGF in peritoneal fluid. In human endometrial

biopsies, VEGF expression increased relative to the early proliferative phase approximately 1.6-, 2-, and 3.6-fold in midproliferative, late proliferative, and secretory endometrium (Shifren *et al.*, 1996, *J. Clin. Endocrinol. Metab.* 81, 3112-3118). In a third study, VEGF-positive staining of human ectopic endometrium was shown to be 5 localized to macrophages (double immunofluorescent staining with CD14 marker). Peritoneal fluid macrophages demonstrated VEGF staining in women with and without endometriosis. However, increased activation of macrophages (acid phosphatase activity) was demonstrated in fluid from women with endometriosis compared with controls. Peritoneal fluid macrophage conditioned media from patients with 10 endometriosis resulted in significantly increased cell proliferation ($[^3\text{H}]$ thymidine incorporation) in HUVEC cells compared to controls. The percentage of peritoneal fluid macrophages with VEGFr2 mRNA was higher during the secretory phase, and significantly higher in fluid from women with endometriosis ($80 \pm 15\%$) compared with controls ($32 \pm 20\%$). Flt-mRNA was detected in peritoneal fluid macrophages from 15 women with and without endometriosis, but there was no difference between the groups or any evidence of cyclic dependence (McLaren *et al.*, 1996, *J. Clin. Invest.* 98, 482-489). In the early proliferative phase of the menstrual cycle, VEGF has been found to be expressed in secretory columnar epithelium (estrogen-responsive) lining both the oviducts and the uterus in female mice. During the secretory phase, VEGF expression 20 was shown to have shifted to the underlying stroma composing the functional endometrium. In addition to examining the endometrium, neovascularization of ovarian follicles and the corpus luteum, as well as angiogenesis in embryonic implantation sites have been analyzed. For these processes, VEGF was expressed in spatial and temporal proximity to forming vasculature (Shweiki *et al.*, 1993, *J. Clin. Invest.* 91, 2235-2243).

25 6) Kidney disease: Autosomal dominant polycystic kidney disease (ADPKD) is the most common life threatening hereditary disease in the USA. It affects about 1:400 to 1:1000 people and approximately 50% of people with ADPKD develop renal failure. ADPKD accounts for about 5-10% of end-stage renal failure in the USA, requiring dialysis and renal transplantation. Angiogenesis is implicated in the progression of 30 ADPKD for growth of cyst cells, as well as increased vascular permeability promoting fluid secretion into cysts. Proliferation of cystic epithelium is a feature of ADPKD because cyst cells in culture produce soluble vascular endothelial growth factor (VEGF).

VEGFr1 has been detected in epithelial cells of cystic tubules but not in endothelial cells in the vasculature of cystic kidneys or normal kidneys. VEGFr2 expression is increased in endothelial cells of cyst vessels and in endothelial cells during renal ischemia-reperfusion.

5 The use of radiation treatments and chemotherapeutics, such as Gemcytabine and cyclophosphamide, are non-limiting examples of chemotherapeutic agents that can be combined with or used in conjunction with the nucleic acid molecules (e.g. siNA molecules) of the instant invention. Those skilled in the art will recognize that other anti-cancer compounds and therapies can similarly be readily combined with the nucleic acid molecules of the instant invention (e.g. siNA molecules) and are hence within the scope of the instant invention. Such compounds and therapies are well known in the art (see for example *Cancer: Principles and Practice of Oncology*, Volumes 1 and 2, eds Devita, V.T., Hellman, S., and Rosenberg, S.A., J.B. Lippincott Company, Philadelphia, USA; incorporated herein by reference) and include, without limitation, folates, 10 antifolates, pyrimidine analogs, fluoropyrimidines, purine analogs, adenosine analogs, topoisomerase I inhibitors, anthrapyrazoles, retinoids, antibiotics, anthacyclins, platinum analogs, alkylating agents, nitrosoureas, plant derived compounds such as vinca alkaloids, epipodophyllotoxins, tyrosine kinase inhibitors, taxols, radiation therapy, surgery, nutritional supplements, gene therapy, radiotherapy, for example 3D-CRT, 15 immunotoxin therapy, for example ricin, and monoclonal antibodies. Specific examples of chemotherapeutic compounds that can be combined with or used in conjunction with the nucleic acid molecules of the invention include, but are not limited to, Paclitaxel; Docetaxel; Methotrexate; Doxorubicin; Edatrexate; Vinorelbine; Tomoxifen; Leucovorin; 5-fluoro uridine (5-FU); Imituzumab; Cisplatin; Carboplatin; Amsacrine; Cytarabine; 20 Bleomycin; Mitomycin C; Dactinomycin; Mithramycin; Hexamethylmelamine; Dacarbazine; L-aspergillase; Nitrogen mustard; Melphalan, Chlorambucil; Busulfan; Ifosfamide; 4-hydroperoxycyclophosphamide; Thiotepa; Irinotecan (CAMPTOSAR®, CPT-11, Camptothecin-11, Campto) Tamoxifen; Herceptin; IMC C225; ABX-EGF; and combinations thereof. The above list of compounds are non-limiting examples of 25 compounds and/or methods that can be combined with or used in conjunction with the nucleic acid molecules (e.g. siNA) of the instant invention. Those skilled in the art will recognize that other drug compounds and therapies can similarly be readily combined 30

with the nucleic acid molecules of the instant invention (e.g., siNA molecules) are hence within the scope of the instant invention.

Example 12: Diagnostic uses

The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple siNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used to inhibit gene expression and define the role of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA molecules and/or other chemical or biological molecules). Other *in vitro* uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siNA using standard methodologies, for example, fluorescence resonance emission transfer (FRET).

In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (*i.e.*, those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present

in the sample and the second siNA molecules (*i.e.*, those that cleave only mutant forms of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence 5 of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection 10 assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, disease related or infection related) is adequate to 15 establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

20 All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

25 One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

30 It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the

scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described herein toward generating nucleic acid constructs with improved 5 activity for mediating RNAi activity. Such improved activity can comprise improved stability, improved bioavailability, and/or improved activation of cellular responses mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested without undue experimentation toward 10 identifying siNA molecules with improved RNAi activity.

The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either 15 of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although 20 the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of 25 Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Table I: VEGF and VEGFr Accession Numbers

5	NM_005429 Homo sapiens vascular endothelial growth factor C (VEGFC), mRNA gi 19924300 ref NM_005429.2 [19924300]
10	NM_003376 Homo sapiens vascular endothelial growth factor (VEGF), mRNA gi 19923239 ref NM_003376.2 [19923239]
15	AF095785 Homo sapiens vascular endothelial growth factor (VEGF) gene, promoter region and partial cds gi 4154290 gb AF095785.1 [4154290]
20	NM_003377 Homo sapiens vascular endothelial growth factor B (VEGFB), mRNA gi 20070172 ref NM_003377.2 [20070172]
25	AF486837 Homo sapiens vascular endothelial growth factor isoform VEGF165 (VEGF) mRNA, complete cds gi 19909064 gb AF486837.1 [19909064]
30	AF468110 Homo sapiens vascular endothelial growth factor B isoform (VEGFB) gene, complete cds, alternatively spliced gi 18766397 gb AF468110.1 [18766397]
35	AF437895 Homo sapiens vascular endothelial growth factor (VEGF) gene, partial cds gi 16660685 gb AF437895.1 AF437895[16660685]
40	AY047581
45	
50	

Homo sapiens vascular endothelial growth factor (VEGF)
mRNA, complete cds
gi|15422108|gb|AY047581.1|[15422108]

5 AF063657
Homo sapiens vascular endothelial growth factor
receptor (FLT1) mRNA, complete
cds
10 gi|3132830|gb|AF063657.1|AF063657[3132830]

AF092127
Homo sapiens vascular endothelial growth factor (VEGF)
15 gene, partial sequence
gi|4139168|gb|AF092127.1|AF092127[4139168]

AF092126
20 Homo sapiens vascular endothelial growth factor (VEGF)
gene, 5' UTR
gi|4139167|gb|AF092126.1|AF092126[4139167]

AF092125
25 Homo sapiens vascular endothelial growth factor (VEGF)
gene, partial cds
gi|4139165|gb|AF092125.1|AF092125[4139165]

E15157
30 Human VEGF mRNA
gi|5709840|dbj|E15157.1||pat|JP|1998052285|2[5709840]

E15156
35 Human VEGF mRNA
gi|5709839|dbj|E15156.1||pat|JP|1998052285|1[5709839]

E14233
40 Human mRNA for vascular endothelial growth factor
(VEGF), complete cds
gi|5708916|dbj|E14233.1||pat|JP|1997286795|1[5708916]

45 AF024710
Homo sapiens vascular endothelial growth factor (VEGF)
mRNA, 3'UTR
50 gi|2565322|gb|AF024710.1|AF024710[2565322]

5 AJ010438
Homo sapiens mRNA for vascular endothelial growth
factor, splicing variant
VEGF183
gi|3647280|emb|AJ010438.1|HSA010438 [3647280]

10 AF098331
Homo sapiens vascular endothelial growth factor (VEGF)
gene, promoter, partial
sequence
gi|4235431|gb|AF098331.1|AF098331 [4235431]

15 AF022375
Homo sapiens vascular endothelial growth factor mRNA,
complete cds
gi|3719220|gb|AF022375.1|AF022375 [3719220]

20 AH006909
vascular endothelial growth factor {alternative
splicing} [human, Genomic, 414
nt 5 segments]
gi|1680143|gb|AH006909.1||bbm|191843 [1680143]

25 U01134
Human soluble vascular endothelial cell growth factor
receptor (sflt) mRNA,
complete cds
gi|451321|gb|U01134.1|U01134 [451321]

30 E14000
Human mRNA for FLT
gi|3252767|dbj|E14000.1||pat|JP|1997255700|1 [3252767]

35 E13332
cDNA encoding vascular endodermal cell growth factor
VEGF
gi|3252137|dbj|E13332.1||pat|JP|1997173075|1 [3252137]

40 E13256
Human mRNA for FLT, complete cds
gi|3252061|dbj|E13256.1||pat|JP|1997154588|1 [3252061]

5 AF063658
 Homo sapiens vascular endothelial growth factor
 receptor 2 (KDR) mRNA, complete
 cds
 gi|3132832|gb|AF063658.1|AF063658 [3132832]

10 AJ000185
 Homo Sapiens mRNA for vascular endothelial growth
 factor-D
 gi|2879833|emb|AJ000185.1|HSAJ185 [2879833]

15 D89630
 Homo sapiens mRNA for VEGF-D, complete cds
 gi|2780339|dbj|D89630.1|[2780339]

20 AF035121
 Homo sapiens KDR/flk-1 protein mRNA, complete cds
 gi|2655411|gb|AF035121.1|AF035121 [2655411]

25 AF020393
 Homo sapiens vascular endothelial growth factor C
 gene, partial cds and 5'
 upstream region
 gi|2582366|gb|AF020393.1|AF020393 [2582366]

30 Y08736
 H.sapiens vegf gene, 3'UTR
 gi|1619596|emb|Y08736.1|HSVEGF3UT [1619596]

35 X62568
 H.sapiens vegf gene for vascular endothelial growth
 factor
 gi|37658|emb|X62568.1|HSVEGF [37658]

40 X94216
 H.sapiens mRNA for VEGF-C protein
 gi|1177488|emb|X94216.1|HSVEGFC [1177488]

45 NM_002020
 Homo sapiens fms-related tyrosine kinase 4 (FLT4),
 mRNA
 gi|4503752|ref|NM_002020.1|[4503752]

5
NM_002253
Homo sapiens kinase insert domain receptor (a type III
receptor tyrosine kinase)
(KDR), mRNA
gi|11321596|ref|NM_002253.1|[11321596]

Table II: VEGFR siNA and Target Sequences

VEGFR1 gi|4503748|ref|NM_002019.1

Pos	Target Sequence	Seq ID	UPos	Upper seq		Seq ID	LPos	Lower seq	Seq ID
				GC	GG				
1	GGGACACUCCUCUGGGCU	1	1	GGGACACUCCUCUGGGCU	1	23	AGCCGAGGGAGGGUCCGC	428	
19	UCCUCCCGGCAGGGGG	2	19	UCCUCCCGGCAGGGGG	2	41	CCGCCGCGUGCCGGGAGGA	429	
37	GCGCUCGGAGGGCUCC	3	37	GCGCUCGGAGGGCUCC	3	59	GGAGCCGGCUCCGAGCCGC	430	
55	CGGGCUCGGUGGAGGGG	4	55	CGGGCUCGGUGGAGGGG	4	77	CCGCUGCAACCCGAGCCCCG	431	
73	GCCAGCGGGCCUGGGGG	5	73	GCCAGCGGGCCUGGGGG	5	95	CGCCGCCAGGGGGCGUGGC	432	
91	GAGGAUUAACCGGGGAAGU	6	91	GAGGAUUAACCGGGGAAGU	6	113	ACUUCCCGGGUAAUCCUC	433	
109	UGGUUGGUCCUGGUGGA	7	109	UGGUUGGUCCUGGUGGA	7	131	UCCAGGCCAGGAGAACCA	434	
127	AGCGCGAGACGGGCU	8	127	AGCGCGAGACGGGCU	8	149	GAGGCCGCGUCUGGGGCU	435	
145	CAGGGCGGGGGGGGG	9	145	CAGGGCGGGGGGGGG	9	167	CGGCGGGGGGGGGGG	436	
163	GGGGCAACGAGAGACGG	10	163	GGGGCAACGAGAGACGG	10	185	CGGUCCUCUCGUUUCGGCGC	437	
181	GACUCUGGGGGGGGUUCG	11	181	GACUCUGGGGGGGGUUCG	11	203	CGACCCGGCCGAGAGUC	438	
199	GUUGGGGGGGGGGGGG	12	199	GUUGGGGGGGGGGGGG	12	221	CGCGCGUCCCCCGGCCAAAC	439	
217	GGCACCGGGGAGGAGGC	13	217	GGCACCGGGGAGGAGGC	13	239	GGCCUGGUCCGGGGUGCC	440	
235	CGCGUCGGCUACCAUGG	14	235	CGCGUCGGCUACCAUGG	14	257	CCAUUUGGAGGCGGACGCG	441	
253	GUAGCUACUGGGACACCG	15	253	GUAGCUACUGGGACACCG	15	275	CGGUUCGCCAGUAGUGAC	442	
271	GGGUUCCUGUGGGCGGC	16	271	GGGUUCCUGUGGGCGGC	16	293	GCGCGCACAGGAGCCCC	443	
289	CUGCUACUGGUCCUUC	17	289	CUGCUACUGGUCCUUC	17	311	GAAGCAGACAGCUGAGCAG	444	
307	CUCACAGGAUCUAGUUCAG	18	307	CUCACAGGAUCUAGUUCAG	18	329	CUGAACUAGAUCCUGUGAG	445	
325	GGUUCAAAUAAGAUC	19	325	GGUUCAAAUAAGAUC	19	347	GAUCUUUUAAAAGAAC	446	
343	CCUGAACUGAGUUAAAAG	20	343	CCUGAACUGAGUUAAAAG	20	365	CUUUAAACUCAGUUCAGG	447	
361	GGCACCCAGCACAUAGC	21	361	GGCACCCAGCACAUAGC	21	383	GCAUGAUGUGUGGGUGGCC	448	
379	CAAGCAGGCCAGACACUG	22	379	CAAGCAGGCCAGACACUG	22	401	GCAGUGUGUGGCCGUUG	449	
397	CAUCUCCAUAGCAGGGGG	23	397	CAUCUCCAUAGCAGGGGG	23	419	CCCCCUUGCAUUGGAGAUG	450	
415	GAAGCAGCCAUAAAUGGU	24	415	GAAGCAGCCAUAAAUGGU	24	437	ACCAUUUAUGGGCUGCUUC	451	
433	UCUUUUGCCUGAAAUGGUGA	25	433	UCUUUUGCCUGAAAUGGUGA	25	455	UCACCAAUUUCAGGCAAAGA	452	
451	AGUAAGGAAAGCGAAAGGC	26	451	AGUAAGGAAAGCGAAAGGC	26	473	GCCUUUCCUUUCCUACU	453	
469	CUGAGCAUAAACUAAAUCUG	27	469	CUGAGCAUAAACUAAAUCUG	27	491	CAGAUUUAGUUUAUGCUAG	454	
487	GCCUGGGAAAGAAUGGCA	28	487	GCCUGGGAAAGAAUGGCA	28	509	UGCCAUUUCUCCACAGGC	455	
505	AAACAAUUCUGCAUACUU	29	505	AAACAAUUCUGCAUACUU	29	527	AAGUACUGCAGAAUUGUU	456	
523	UUACCUUGAACACAGCUC	30	523	UUACCUUGAACACAGCUC	30	545	GAGCUGUGUUCAAGGUAA	457	

541	CAAGCAAACCACUGGCC	31	541	CAAGCAAACCACUGGCC	31	563	AGCCAGUGUGGUUGGUUG	458
559	UUUCUACAGCGUGCAAAUUC	32	559	UUUCUACAGCGUGCAAAUUC	32	581	GAUAUUUGCGCUGUAGAA	459
577	CUAGCGUGUACCUACUCAA	33	577	CUAGCGUGUACCUACUCAA	33	599	UJGAAGUAGGUACAGCGUAG	460
595	AAGAAAGAAGGAAACAGAAU	34	595	AAGAAAGAAGGAAACAGAAU	34	617	AUUCUGUUCUCCUUCUUCU	461
613	UCUGCAAUCUAAUUAUUA	35	613	UCUGCAAUCUAAUUAUUA	35	635	UAAAUAUAUAGAUUGCAGA	462
631	AUUAUGUGAUACAGGUAGAC	36	631	AUUAUGUGAUACAGGUAGAC	36	653	GUCUACCUGUUAUCACUAAU	463
649	CCUUUCGUAGAGAUGUACA	37	649	CCUUUCGUAGAGAUGUACA	37	671	UGUACAUUCUACGAAAGG	464
667	AGUGAAAUCCCGAAAUUA	38	667	AGUGAAAUCCCGAAAUUA	38	689	UAAAUUUGGGAUUUCACU	465
685	AUACACAUAGACUGAAGGAA	39	685	AUACACAUAGACUGAAGGAA	39	707	UCCCUUCAGUCAUGUGUAU	466
703	AGGGAGGCUGGUCAUCCU	40	703	AGGGAGGCUGGUCAUCCU	40	725	AGGGAAUAGCAGGCUCCCU	467
721	UGCCGGGUACGUACCUA	41	721	UGCCGGGUACGUACCUA	41	743	UAGGGAGGUACCCGGCA	468
739	AACAUACUGUUAUCUUA	42	739	AACAUACUGUUAUCUUA	42	761	UAAAUGUAACAGUGAUGUU	469
757	AAAAGUJJUCCACUUGACA	43	757	AAAAGUJJUCCACUUGACA	43	779	UGUCAAGUGGAAACUUUUU	470
775	ACUUUGAUCCCCUGAUGGAA	44	775	ACUUUGAUCCCCUGAUGGAA	44	797	UCCCAUCAGGGAUCAAAAGU	471
793	AAACGCCAAUACUGGGACA	45	793	AAACGCCAAUACUGGGACA	45	815	UGUCCCCAGAUUAGCGUUU	472
811	AGUAGAAAAGGGCUUCAUCA	46	811	AGUAGAAAAGGGCUUCAUCA	46	833	UGAUGAAGGCCCUUUCUACU	473
829	AUAUCAAAUUGCAACGUACA	47	829	AUAUCAAAUUGCAACGUACA	47	851	UGUACGUUGCAUUGUAU	474
847	AAAGAAAUAAGGGCUUCUGA	48	847	AAAGAAAUAAGGGCUUCUGA	48	869	UCAGAAGGCCCUAUUUCUUU	475
865	ACCUGUGAAGCAACAGUCA	49	865	ACCUGUGAAGCAACAGUCA	49	887	UGACUGUUGCUUACAGGU	476
883	AAUGGGCAUUUGUAUAGA	50	883	AAUGGGCAUUUGUAUAGA	50	905	UCUUUAUACAAAUGGCCAUU	477
901	ACAAAACUACUACACACAUC	51	901	ACAAAACUACUACACACAUC	51	923	GAUGUGUGAGAUAGUUGU	478
919	CGACAAACCAAAUCAAUCA	52	919	CGACAAACCAAAUCAAUCA	52	941	UGAUJGUAUUGGUUGUUCG	479
937	AUAGAUGUCCAAAUAAGCA	53	937	AUAGAUGUCCAAAUAAGCA	53	959	UGCUUUAUJUGGACAUUCUAU	480
955	ACACCCACGCCAGUAAA	54	955	ACACCCACGCCAGUAAA	54	977	AUJUGACUGGGCGUGGGUG	481
973	UUACUUAGGGCCAUACUC	55	973	UUACUUAGGGCCAUACUC	55	995	GAGUAGGGCCUCUAAGUAA	482
991	CUUGGUCCUAAUUGUACUG	56	991	CUUGGUCCUAAUUGUACUG	56	1013	CAGUACAAUUGAGGACAAG	483
1009	GCUUACCAUCCCUUUAACA	57	1009	GCUUACCAUCCCUUUAACA	57	1031	UGUUCAAGGGAGUGGUAGC	484
1027	ACGAGAGUCAAAGGACCU	58	1027	ACGAGAGUCAAAGGACCU	58	1049	AGGUCAUUIUGAACUCUCGU	485
1045	UGGAGUUACCCUGAUGAAA	59	1045	UGGAGUUACCCUGAUGAAA	59	1067	UUUCUACAGGGGUACUCCCA	486
1063	AAAAAAUAGAGAGCUUCCG	60	1063	AAAAAAUAGAGAGCUUCCG	60	1085	CGGAAGCUCUCUUAUUUUU	487
1081	GUAGGGCAGCAUJUGACC	61	1081	GUAGGGCAGCAUJUGACC	61	1103	GGCUAAUUCGUCGCUUAC	488
1099	CAAAGCAAUCCCAUGCCA	62	1099	CAAAGCAAUCCCAUGCCA	62	1121	UGGCAUGGGAAUUGUUUUG	489
1117	AACAUAUUCUACAGUUC	63	1117	AACAUAUUCUACAGUUC	63	1139	GAACACUGUAGAAUAUGUU	490
1135	CUUACUAAUUGACAAAUGC	64	1135	CUUACUAAUUGACAAAUGC	64	1157	GCAUUUUUGCUAAUAGUAAG	491
1153	CAGAACAAAGACAAAGGAC	65	1153	CAGAACAAAGACAAAGGAC	65	1175	GUCCUUUUGCUUUUGUUCUG	492
1171	CUUUAUACUUGGUUGUAA	66	1171	CUUUAUACUUGGUUGUAA	66	1193	UUACACGACAAGUAUAAAG	493

1189	AGGAGUGGACCAUCAUCA	67	1189	AGGAGUGGACCAUCAUCA	67	1211	UGAAUGAUGGUCCACUCCU	494
1207	AAUCUGUUAAACCCUCAG	68	1207	AAALCUGUUAAACCCUCAG	68	1229	CUGAGGUGUUACAGAUUU	495
1225	GUGCAUAAUUAUGAUAAAG	69	1225	GUGCAUAAUUAUGAUAAAG	69	1247	CUUUAUCAUAAUUAUGCAC	496
1243	GCAUUCACUCUGUGAAC	70	1243	GCAUUCACUCUGUGAAC	70	1265	GUUUACAGUGAUGAAUGC	497
1261	CAUCGAAAACAGCAGGUUC	71	1261	CAUCGAAAACAGCAGGUUC	71	1283	GCACCUGCUGUUUUCGAUG	498
1279	CUUGAAACCGUAGCUGGCA	72	1279	CUUGAAACCGUAGCUGGCA	72	1301	UGCCAGCUACGGUUUUCAG	499
1297	AAGCGGUUUACCGGCUU	73	1297	AAGCGGUUUACCGGCUU	73	1319	AGAGCGGGUUAAGACCGCUU	500
1315	UCUAUGAAAGUGAAGGCAU	74	1315	UCUAUGAAAGUGAAGGCAU	74	1337	AUGGCCUUACUUUCAUAGA	501
1333	UJUCCCUUGCCGGAAGUUG	75	1333	UJUCCCUUGCCGGAAGUUG	75	1355	CAACUUCGGGGAGGGAAA	502
1351	GUAUUGUUAAAAGAUGGGU	76	1351	GUAUUGUUAAAAGAUGGGU	76	1373	ACCCAUCUUUUAACCAUAC	503
1369	UUACCUGGACUGAGAAAUA	77	1369	UUACCUGGACUGAGAAAUA	77	1391	AUUUCUCAGUCGCGAGGUAA	504
1387	UCUGCUCCUUAUUGACUC	78	1387	UCUGCUCCUUAUUGACUC	78	1409	GAGUCUAAUAGCGAGCAGA	505
1405	CGUGGCUACUCGUUAUUA	79	1405	CGUGGCUACUCGUUAUUA	79	1427	UAAUUAACCGAGUAGGCCACG	506
1423	AUCAAGGGACGUACUGAAG	80	1423	AUCAAGGGACGUACUGAAG	80	1445	CUUCAGUUACGUCCUUGAU	507
1441	GAGGAUGCAGGGAAUUAUA	81	1441	GAGGAUGCAGGGAAUUAUA	81	1463	UAAAUIUCCCUGCAUCCUC	508
1459	ACAAACUUCUGCUGAGCAUAA	82	1459	ACAAACUUCUGCUGAGCAUAA	82	1481	UUAUGCUUCAGCAAGAUUU	509
1477	AAACAGGUCAAAGUGUUUA	83	1477	AAACAGGUCAAAGUGUUUA	83	1499	UAAAACACAUUUGACUGUUU	510
1495	AAAAAACCUACUGCCACUC	84	1495	AAAAAACCUACUGCCACUC	84	1517	GAGGGCAGUGAGGUUUUU	511
1513	CUAAUUGUCAAUGUGAAAC	85	1513	CUAAUUGUCAAUGUGAAAC	85	1535	GUUUACACAUUAGCAAAUAG	512
1531	CCCCAGAUUUACGAAAAGG	86	1531	CCCCAGAUUUACGAAAAGG	86	1553	CCUUUUUCGUAAAUCUGGGG	513
1549	GGCGUGUGCAUCGUUUCAG	87	1549	GGCGUGUGCAUCGUUUCAG	87	1571	CUGGAAACGAUGACACGGC	514
1567	GACCCGGCUCUCUACCCAC	88	1567	GACCCGGCUCUCUACCCAC	88	1589	GUGGGUAGAGGCGGGGUC	515
1585	CUGGGCAGCAGACAAUCC	89	1585	CUGGGCAGCAGACAAUCC	89	1607	GGAUUUIUGCUGCUGCCAG	516
1603	CUGACUUGUACCGCAUAG	90	1603	CUGACUUGUACCGCAUAG	90	1625	CAUAGCGGUACAGUGCAG	517
1621	GGUAUCCCUCAACCUCAA	91	1621	GGUAUCCCUCAACCUCAA	91	1643	UUGUAGGUUGAGGGAUACC	518
1639	AUCAAGUGGUUCUGGCACC	92	1639	AUCAAGUGGUUCUGGCACC	92	1661	GGUGCCAGAAACCUUGAU	519
1657	CCCUUGUAACCAUAAUCAUU	93	1657	CCCUUGUAACCAUAAUCAUU	93	1679	AUAUGAUUAUGGUUACAGGG	520
1675	UCCGGAAGCAAGGUGUGACU	94	1675	UCCGGAAGCAAGGUGUGACU	94	1697	AGUCACACCUUGCUUCGGGA	521
1693	UUUUGUUCCAAUAAAUGAAG	95	1693	UUUUGUUCCAAUAAAUGAAG	95	1715	CUUCAUUAUUGGAACAAA	522
1711	GAGGUCCUUUACCUUGGAUG	96	1711	GAGGUCCUUUACCUUGGAUG	96	1733	CAUCCAGGUAUAGGACUC	523
1729	GCUGACAGCAACAUUGGAA	97	1729	GCUGACAGCAACAUUGGAA	97	1751	UCCCCAUUGUUGCUGUCAGC	524
1747	AACAGAAUJUGAGGCAUCA	98	1747	AACAGAAUJUGAGGCAUCA	98	1769	UGAUGCUUCUCAUUCUGUU	525
1765	ACUCAGGGCAUGGCAAAUA	99	1765	ACUCAGGGCAUGGCAAAUA	99	1787	UUAUUGCCAUGGGCUGAGU	526
1783	AUAGAAAGGAAAAGAAUAGA	100	1783	AUAGAAAGGAAAAGAAUAGA	100	1805	UCUUAUCUUCUUCUUCUAU	527
1801	AUGGCUAGGCACCUUGGUUG	101	1801	AUGGCUAGGCACCUUGGUUG	101	1823	CAACCAAGGUGCUAGGCCAU	528
1819	GUGGCUGACUCUAGAAUUU	102	1819	GUGGCUGACUCUAGAAUUU	102	1841	AAAUCUJAGAGGCCAC	529

1837	UCUGGAALCUACAUUUGCA	103	1837	UCUGGAAUCUACAUUUGCA	103	1859	UGCAAAUGUAGAUUCCAGA	530
1855	AUAGCUUCCAAUAGGUUG	104	1855	AUAGCUUCCAAUAGGUUG	104	1877	CAACUUUUAUGGAAGCUAU	531
1873	GGGACUGGGGAAGAACAA	105	1873	GGGACUGGGGAAGAACAA	105	1895	UGUUUUCUUCCCACAGUCCC	532
1891	AUAAGCUUUAUACACAG	106	1891	AUAAGCUUUAUACACAG	106	1913	CUGUGAUAAAAGCUUAU	533
1909	GAUGUGCCAAUGGGUUUC	107	1909	GAUGUGCCAAUGGGUUUC	107	1931	GAAACCCAUUJGGCACACU	534
1927	CAUGUUAACUUGGAAAAAA	108	1927	CAUGUUAACUUGGAAAAAA	108	1949	UUUUUCCAAAGUAAACAU	535
1945	AUGCCGAOGGAAGGAGGG	109	1945	AUGCCGAOGGAAGGAGGG	109	1967	CCUCUCUUCCGUCCGGCAU	536
1963	GACCUGAAACUGCUUGCA	110	1963	GACCUGAAACUGCUUGCA	110	1985	UGCAAGACAGUUUCAGGUC	537
1981	ACAGUUAACAAAGUUCUAU	111	1981	ACAGUUAACAAAGUUCUAU	111	2003	AUAAGAACUUGUUAACUGU	538
1999	UACAGAGAGGUUACUUGGA	112	1999	UACAGAGAGGUUACUUGGA	112	2021	UCCAAGUAAACGUUCUCUGUA	539
2017	AUUUUACUGGGACAGUUA	113	2017	AUUUUACUGGGACAGUUA	113	2039	UAAUCUGUCCCGAGUAAAUAU	540
2035	AAUAAACAGAACAAUGCACU	114	2035	AAUAAACAGAACAAUGCACU	114	2057	AGUGCAUUGUUUCUGUUUAU	541
2053	UACAGUAUAGCAAGCAA	115	2053	UACAGUAUAGCAAGCAA	115	2075	UUUGCUGCUAAUACUGUA	542
2071	AAAAUUGGCCAUACUAAGG	116	2071	AAAAUUGGCCAUACUAAGG	116	2093	CCUUAGUGAUAGGCCAUUUU	543
2089	GAGCACUCCAUACUCUUA	117	2089	GAGCACUCCAUACUCUUA	117	2111	UAAGAGUGAUGGGAGUGCUC	544
2107	AAUCUUAACCAUCAUGAAUG	118	2107	AAUCUUAACCAUCAUGAAUG	118	2129	CAUCAUGAUAGGUUAAGAU	545
2125	GUUUCUCUGCAAGAUUCAG	119	2125	GUUUCUCUGCAAGAUUCAG	119	2147	CUGAAUCUUGCAGGGAAAC	546
2143	GGCACCUAUGCCUGCAGAG	120	2143	GGCACCUAUGCCUGCAGAG	120	2165	CUCUGCAGGCCAUAGGGUGCC	547
2161	GCCAGGAUAGUAUACACAG	121	2161	GCCAGGAUAGUAUACACAG	121	2183	CUGUGUAUACAUUCUGGC	548
2179	GGGGAAAGAAUCCUCCAGA	122	2179	GGGGAAAGAAUCCUCCAGA	122	2201	UCUGGAGGAAUUCUCCCC	549
2197	AAGAAAAGAAUACAUCA	123	2197	AAGAAAAGAAUACAUCA	123	2219	UGAUUGUAAAUCUUUUUU	550
2215	AGAGAUCAAGAACCCAU	124	2215	AGAGAUCAAGAACCCAU	124	2237	AUGGUGCUUCCUGAUACUCU	551
2233	UACCUUCUCCGGAACCUCA	125	2233	UACCUUCUCCGGAACCUCA	125	2255	UGAGGUUUCGAGGAGGUA	552
2251	AGUGAUCAACAGUGGCCA	126	2251	AGUGAUCAACAGUGGCCA	126	2273	UGGCCACUGUGUAGAUACU	553
2269	AUCAGCAUUCACCACUU	127	2269	AUCAGCAUUCACCACUU	127	2291	AAGUGGGGAACUGUGUGAU	554
2287	UUAGACUGUCAUGCUAAUG	128	2287	UUAGACUGUCAUGCUAAUG	128	2309	CAUAGCAUGACAGUCUAA	555
2305	GGGUGUCCCGAGCCUCAGA	129	2305	GGGUGUCCCGAGCCUCAGA	129	2327	UCUGGGCUCGGGAGCACCC	556
2323	AUCACUJGGUUAAAACA	130	2323	AUCACUJGGUUAAAACA	130	2345	UGUIUUAAAACCAAGUGAU	557
2341	AACCACAAAUAACAAAG	131	2341	AACCACAAAUAACAAAG	131	2363	CUUGUUGUUUUUGGGUU	558
2359	GAGCCUGAAUUAUJUAG	132	2359	GAGCCUGAAUUAUJUAG	132	2381	CUAAAUAUUCAGGGCUC	559
2377	GGACCAAGGAAGCACGC	133	2377	GGACCAAGGAAGCACGC	133	2399	GCGUGUGCUUCCUGGUCC	560
2395	CUGUUUAUJGAAAGAGUCA	134	2395	CUGUUUAUJGAAAGAGUCA	134	2417	UGACUUCUUCAUAAAACAG	561
2413	ACAGAAGAGGAAGGAGG	135	2413	ACAGAAGAGGAAGGAGG	135	2435	CACCUUCUCCUCUUCUGU	562
2431	GUCUAUCACUGCAAAGCCA	136	2431	GUCUAUCACUGCAAAGCCA	136	2453	UGGCUUUCGAGUAGAGAC	563
2449	ACCAACCAGAAGGGCUCUG	137	2449	ACCAACCAGAAGGGCUCUG	137	2471	CAGAGCCCUCUGGUUGGU	564
2467	GUGGAAAGGUUCAGCAUACC	138	2467	GUGGAAAGGUUCAGCAUACC	138	2489	GGUAUGCUGAACUUCAC	565

2485	CUCACUGUUCAAGGAACCU	139	2485	CUCACUGUUCAAGGAACCU	139	2507	AGGUUCCUUGAACAGUGAG	566
2503	UCGGACAAGGUAAAUCUGG	140	2503	UCGGACAAGGUAAAUCUGG	140	2525	CCAGAUUAGACUUGGUCCGA	567
2521	GAAGCUGAUCACUCAACAU	141	2521	GAGCUGAUCACUCAACAU	141	2543	AUGUUAGAGUGAACAGCUC	568
2539	UGCACCUGUGGGCUCGGA	142	2539	UGCACCUGUGGGCUCGGA	142	2561	UCGCAGGCCACACAGGUGCA	569
2557	ACUCUCUUCCUGGUCCUUAU	143	2557	ACUCUCUUCCUGGUCCUUAU	143	2579	AUAGGAGGCCAGAACAGAGAU	570
2575	UUAACCCUCCUUAUCCGAA	144	2575	UUAACCCUCCUUAUCCGAA	144	2597	UUCGGAUAGGGGGUUAA	571
2593	AAAAUGAAAAGGUUCUUCU	145	2593	AAAAUGAAAAGGUUCUUCU	145	2615	AAGAAAGACCUUUUCAUUUU	572
2611	UCUGAAAUAAGACUAGACU	146	2611	UCUGAAAUAAGACUAGACU	146	2633	AGUCAGUCUUUUUUCAGA	573
2629	UACCUUAUCAAUUAUAAUGG	147	2629	UACCUUAUCAAUUAUAAUGG	147	2651	CCAUUAUAAAUGAUAGGUA	574
2647	GAACCCAGAUGAAGGUCCU	148	2647	GAACCCAGAUGAAGGUCCU	148	2669	AAGGAACUUCAUCCUGGGUC	575
2665	UJGGGAUGAGCAGUGGAGC	149	2665	UJGGGAUGAGCAGUGGAGC	149	2687	GCUCACACUGCUCAUCCAA	576
2683	CGGCUCUCCUUAUGAUCCCA	150	2683	CGGCUCUCCUUAUGAUCCCA	150	2705	UGGCAUCAUAAAGGGAGGCC	577
2701	AGCAAGUGGGAGUUUUCCCC	151	2701	AGCAAGUGGGAGUUUUCCCC	151	2723	GGGCAAAACUCCCAUCUJGGCU	578
2719	CGGGAGAGACUUAACUGG	152	2719	CGGGAGAGACUUAACUGG	152	2741	CCAGUUUAAGUCUCUCCCCG	579
2737	GGCAAAAUCAUCUUGGAAGAG	153	2737	GGCAAAAUCAUCUUGGAAGAG	153	2759	CUCUUCCAAGUGAUUUJGCC	580
2755	GGGGCUUUUJGGAAAAGUGG	154	2755	GGGGCUUUUJGGAAAAGUGG	154	2777	CCACUUIUUCCAAAAGCCCC	581
2773	GUUCAAGGAUCAGCAUUUJ	155	2773	GUUCAAGGAUCAGCAUUUJ	155	2795	CAAAUGCUGAUGCUJUGAAC	582
2791	GGCAUUAGAAAUCACCUA	156	2791	GGCAUUAGAAAUCACCUA	156	2813	UAGGUGAUUUUCUUAUGCC	583
2809	ACGUGGCCGACUGUGGCCUG	157	2809	ACGUGGCCGACUGUGGCCUG	157	2831	CAGGCCACAGUCGGCAGCGU	584
2827	GUAGAAAAGUCUGAAAAGAGG	158	2827	GUAGAAAAGUCUGAAAAGAGG	158	2849	CCUCUUCAGCAUJJUCAC	585
2845	GGGGCCACGGCCAGGAGU	159	2845	GGGGCCACGGCCAGGAGU	159	2867	ACUJGGUGGCCUGGGCCCC	586
2863	UACAAAGCUCUGAUGACUG	160	2863	UACAAAGCUCUGAUGACUG	160	2885	CAGUCAUCAAGGCUUUJUGUA	587
2881	GAACUAAAUCUUGACCC	161	2881	GAACUAAAUCUUGACCC	161	2903	GGGUCAAGAUUUUJAGCUC	588
2899	CACAUUGGCCACCAUCUGA	162	2899	CACAUUGGCCACCAUCUGA	162	2921	UCAGAUGGUGGCCAAUGUG	589
2917	AACGUGGUAAAUCUGUGG	163	2917	AACGUGGUAAAUCUGUGG	163	2939	CCAGCAGGUUAACCACCGUU	590
2935	GGAGGCCUGACCAAGCAAG	164	2935	GGAGGCCUGACCAAGCAAG	164	2957	CUUGCUCUGGCGAGGCCUCC	591
2953	GGAGGGCCUCUGAUGGUGA	165	2953	GGAGGGCCUCUGAUGGUGA	165	2975	UCACCAUCAGGGGCCCUCC	592
2971	AUUGUUJGAAUACUGAAAAU	166	2971	AUUGUUJGAAUACUGAAAAU	166	2993	AUUGGAGAUUUCACAAAU	593
2989	UAUGGAAAUCUCUCCAAU	167	2989	UAUGGAAAUCUCUCCAAU	167	3011	AGUUGGAGAGAUUCCAAUA	594
3007	UACCUCAAGGCAAACGGUG	168	3007	UACCUCAAGGCAAACGGUG	168	3029	CACGCUUIGCUCUJUGGUA	595
3025	GAUCUAAAUCUUCUCAACA	169	3025	GAUCUAAAUCUUCUCAACA	169	3047	UGUUGAGAAAAAAJAGUC	596
3043	AAGGAUGCAAGCACUACACA	170	3043	AAGGAUGCAAGCACUACACA	170	3065	UGUGUAGUGCUGCAUCUU	597
3061	AUGGAGGCCUAGAAAAGAAA	171	3061	AUGGAGGCCUAGAAAAGAAA	171	3083	UUUCUUCUJUGGCCUCAU	598
3079	AAAUAUGGAGGCCAGGCCUGG	172	3079	AAAUAUGGAGGCCAGGCCUGG	172	3101	CCAGGCCUGGCCUCAUUU	599
3097	GAACAAAGGCAAGAAAACCA	173	3097	GAACAAAGGCAAGAAAACCA	173	3119	UJGGGUUCUUGGCCUJGUUC	600
3115	AGACUAGAUAGGGUCACCA	174	3115	AGACUAGAUAGGGUCACCA	174	3137	UGGUGACGCUAUCAUAGUCU	601

3133	AGCAGCGAAAGCUUUCGCA	175	3133	AGCAGCGAAAGCUUUCGCA	175	3155	UCGCAAAGCUUUCGCGCU	602
3151	AGCUCCGGCUUUCAGGAAG	176	3151	AGCUCCGGCUUUCAGGAAG	176	3173	CUUCCUGAAAGCCGGAGCU	603
3169	GAUAAAAGUCUGAGUGAUG	177	3169	GAUAAAAGUCUGAGUGAUG	177	3191	CAUCACUCAGACUUUAUC	604
3187	GUUGAGGAAGAGGGAGAUU	178	3187	GUUGAGGAAGAGGGAGAUU	178	3209	AAUCCUCCUUCUCCUAC	605
3205	UCUGACGGUUUCUACAAGG	179	3205	UCUGACGGUUUCUACAAGG	179	3227	CCUUGUAGAAACCGUCAGA	606
3223	GAGGCCAUACUAUGGAAG	180	3223	GAGGCCAUACUAUGGAAG	180	3245	CUUCCAUAGUGAUGGGCUC	607
3241	GAUCUGAUUUCUJACAGUU	181	3241	GAUCUGAUUUCUJACAGUU	181	3263	AACUGUAGAAAUCAGAUC	608
3259	UUUCAAGUGGCCAGAGCA	182	3259	UUUCAAGUGGCCAGAGCA	182	3281	UGCCUCUGGCCACUJUGAAA	609
3277	AUGGAGGUCCUGCUUCCA	183	3277	AUGGAGGUCCUGCUUCCA	183	3299	UGGAAGACAGGAACUCUCAU	610
3295	AGAAAGUGCAUUCAGGG	184	3295	AGAAAGUGCAUUCAGGG	184	3317	CCCGAUGAAUUGCAUCUUUC	611
3313	GAACUJGGCAAGCGAGAAACA	185	3313	GAACUJGGCAAGCGAGAAACA	185	3335	UGUUUUUCJCGCUGCCAGGU	612
3331	AUUCUUUUUACUGAGAAC	186	3331	AUUCUUUUUACUGAGAAC	186	3353	UGUUUCUCAGAUAAAAGAAU	613
3349	AACGUGGGUGAAAGUUJUG	187	3349	AACGUGGGUGAAAGUUJUG	187	3371	CACAAAUUCUUCACCAAGGU	614
3367	GAUUUUGGCCUUGCCGG	188	3367	GAUUUUGGCCUUGCCGG	188	3389	CCGGGGCAAGGCCAAAUC	615
3385	GAUAUUUUAAGAACCCG	189	3385	GAUAUUUUAAGAACCCG	189	3407	CGGGGUUCUUAUAAAUAUC	616
3403	GAUUAUGUGAGAAAAGGAG	190	3403	GAUUAUGUGAGAAAAGGAG	190	3425	CUCCUUUUUCACAUAAAUC	617
3421	GAUACUCGACUUCUCUGA	191	3421	GAUACUCGACUUCUCUGA	191	3443	UCAGAGGAAGUGCGAGUAUC	618
3439	AAAUGGAUGGCCUCCGAAU	192	3439	AAAUGGAUGGCCUCCGAAU	192	3461	AUUCGGGAGGCCAUCCAUU	619
3457	UCUAUCUUUUGACAAAUCU	193	3457	UCUAUCUUUUGACAAAUCU	193	3479	AGAUUUJUGCUAAAGAUAGA	620
3475	UACAGGCACCAAGAGCGACG	194	3475	UACAGGCACCAAGAGCGACG	194	3497	CGUCGCUCUUCUUGGU	621
3493	GUUGGGUCUUACGGGUAU	195	3493	GUUGGGUCUUACGGGUAU	195	3515	AUACUCGGUAAGACCACAC	622
3511	UUGGCGUGGGAAAUCUUCU	196	3511	UUGGCGUGGGAAAUCUUCU	196	3533	AGAAGAUUUUCACAGCAA	623
3529	UCCUUAGGGGGUCUCCAU	197	3529	UCCUUAGGGGGUCUCCAU	197	3551	AUGGAGACCCACCUAGGA	624
3547	UACCCAGGAGUACAAAUGG	198	3547	UACCCAGGAGUACAAAUGG	198	3569	CCAUUUUGACUCCUGGGUA	625
3565	GAUGAGGACUUUJUGCAUC	199	3565	GAUGAGGACUUUJUGCAUC	199	3587	GACUGCAAAAGUCUCUCAUC	626
3583	CGCCUGAGGGAAAGCAUGA	200	3583	CGCCUGAGGGAAAGCAUGA	200	3605	UCAUGCCUUCUCCUCAGGG	627
3601	AGGAUGAGAGCUCCUGAGU	201	3601	AGGAUGAGAGCUCCUGAGU	201	3623	ACUCAGGAGCUCUCAUCU	628
3619	UACUCUACUCCUGAAAUCU	202	3619	UACUCUACUCCUGAAAUCU	202	3641	AGAUUUUCAGGAGUAGAGUA	629
3637	UAUCAGAUCAUGCGGACU	203	3637	UAUCAGAUCAUGCGGACU	203	3659	AGUCCAGCAUGAUCUGAU	630
3655	UGCUGGGCACAGAGCCAA	204	3655	UGCUGGGCACAGAGCCAA	204	3677	UGGGGUCUCUGGCCAGCA	631
3673	AAAGAAAAGGCCAAGUUUJ	205	3673	AAAGAAAAGGCCAAGUUUJ	205	3695	CAAUCUJUGGCCUJUUUU	632
3691	GCAGAACUJUGGGAAAAC	206	3691	GCAGAACUJUGGGAAAAC	206	3713	GUUUUUCCACAGUUCUGC	633
3709	CUAGGUGAUUUGCUUCAAG	207	3709	CUAGGUGAUUUGCUUCAAG	207	3731	CUUGAAGCAAAUCACCUC	634
3727	GCAAAAGUACAACAGGAUG	208	3727	GCAAAAGUACAACAGGAUG	208	3749	CAUCCUGUUGUACUUUGC	635
3745	GUAAAAGACUACAUCCAA	209	3745	GUAAAAGACUACAUCCAA	209	3767	UJGGGAUGUAGUACUUC	636
3763	AUCAAUGCCAUCUGACAG	210	3763	AUCAAUGCCAUCUGACAG	210	3785	CUGUCAGUAGUGCAUJUGAU	637

3781	GGAAAUGUGGGUUUACAU	211	3781	GGAAAUAUGGGUUUACAU	211	3803	AUGUAAAACCCACAUUUUCC	638
3799	UACUCAAUCUCCUGCCUUCU	212	3799	UACUCAAACUCCUGCCUUCU	212	3821	AGAAGGCAGGGAGUJAGUA	639
3817	UCUGAGGACUUCUUCAGG	213	3817	UCUGAGGACUUCUUCAGG	213	3839	CCUUGAAAGGUCCUCAGA	640
3835	GAAAGGUUUUCAGCUCCGA	214	3835	GAAAGGUUUUCAGCUCCGA	214	3857	UCGGAGCUGAAAUACUUUC	641
3853	AAGGUUUUUUCAGGAAGCU	215	3853	AAGGUUUUUUCAGGAAGCU	215	3875	AGCUUCCUGAAUAAAACUU	642
3871	UCUGAUGAUGUCAGAU AUG	216	3871	UCUGAUGAUGUCAGAU AUG	216	3893	CAUAUCUGACAUCAUCAGA	643
3889	GUAAAUGCUUUCAAGUUCA	217	3889	GUAAAUGCUUUCAAGUUCA	217	3911	UGAACUJUGAAAAGCAUUUAC	644
3907	AUGAGGCCUGGAAAAGAUCA	218	3907	AUGAGGCCUGGAAAAGAUCA	218	3929	UGAUUUCUJUUCAGGCCUCAU	645
3925	AAAACCUUUGAAGAACUUU	219	3925	AAAACCUUUGAAGAACUUU	219	3947	AAAGUUCUUCAAAGGUUUU	646
3943	UUACCGAAUUGCCACCUCA	220	3943	UUACCGAAUUGCCACCUCA	220	3965	UGGAGGUJGGCAUUCGGGUAA	647
3961	AUGUUJUGAUGACUACAGG	221	3961	AUGUUJUGAUGACUACAGG	221	3983	CCUGGUAGUCUCAAAACAU	648
3979	GGCGACAGCAGCACUCUGU	222	3979	GGCGACAGCAGCACUCUGU	222	4001	ACAGAGGUCCUGGUCCGCC	649
3997	UUGGCCUCUCCCCAUGCUGA	223	3997	UUGGCCUCUCCCCAUGCUGA	223	4019	UCAGCAUGGGAGAGGCCAA	650
4015	AAGGCGCUUUCACCUGGACUG	224	4015	AAGGCGCUUUCACCUGGACUG	224	4037	CAGUCCAGGUAGGCGCUU	651
4033	GACAGGCAAACCCAAAGGCCU	225	4033	GACAGGCAAACCCAAAGGCCU	225	4055	AGGCCUUCUGGUUJUGCUGUC	652
4051	UCGCUCUAAGAUUGACUUGA	226	4051	UCGCUCUAAGAUUGACUUGA	226	4073	UCAAGUCAAUUCUUGAGCGGA	653
4069	AGAGUAAACAGUAAAAGUA	227	4069	AGAGUAAACAGUAAAAGUA	227	4091	UACUUUUACUGGUJACUCU	654
4087	AGGGAGUCCGGGCGUGUCUG	228	4087	AGGGAGUCCGGGCGUGUCUG	228	4109	CAGACAGCCCCGACUCUUU	655
4105	GAUGUCAGCAGGGCCAGUU	229	4105	GAUGUCAGCAGGGCCAGUU	229	4127	AAUCGGGCCUGGUJAGACAUC	656
4123	UUCUGCCAUUCCAGCUGUG	230	4123	UUCUGCCAUUCCAGCUGUG	230	4145	CACAGCUGGAAUUGCAGAA	657
4141	GGGCACGGUACGGGAAGGCA	231	4141	GGGCACGGUACGGGAAGGCA	231	4163	UGCCUUCGGCGUACGGUGGCC	658
4159	AAGGCGAGGUUCACCUACG	232	4159	AAGGCGAGGUUCACCUACG	232	4181	CGUAGGUAGACCUGGCCU	659
4177	GAACCACGCGUGAGCUGAAA	233	4177	GAACCACGCGUGAGCUGAAA	233	4199	UUCCAGCUCAGCGUGGGUC	660
4195	AGGAAAAAUCCGGUGUGCCU	234	4195	AGGAAAAAUCCGGUGUGCCU	234	4217	AGCAGCAACCGCAUUUCCU	661
4213	UCCCCGCCAGACUACA	235	4213	UCCCCGCCAGACUACA	235	4235	UGUAGUCUGGGGGGGGA	662
4231	AACUCUGGGGUCCUGUACU	236	4231	AACUCUGGGGUCCUGUACU	236	4253	AGUACAGGGACCCGGAGUU	663
4249	UCCACCCACCAUCUJAGA	237	4249	UCCACCCACCAUCUJAGA	237	4271	UCUAGAUGGGGGGGGGUGGA	664
4267	AGUUUGACAGCAAGCCUUA	238	4267	AGUUUGACAGCAAGCCUUA	238	4289	UAAGGGCUUCGGGUAAAACU	665
4285	AUUUCUJAGAAGCAGCAGUG	239	4285	AUUUCUJAGAAGCAGCAGUG	239	4307	CACAGUGGUUCUAGAAAAU	666
4303	GUAUUJUAACCCCAGGAA	240	4303	GUAUUJUAACCCCAGGAA	240	4325	UUCUGGGGGGUAAAACUAC	667
4321	ACAUAGCUUUJUGCCAGUAU	241	4321	ACAUAGCUUUJUGCCAGUAU	241	4343	AUACUGGCAAAAGCUAGUU	668
4339	UUAUGCAUUAUAAGUUUA	242	4339	UUAUGCAUUAUAAGUUUA	242	4361	UAAACUJUAUAUAUGCAUUA	669
4357	ACACCUUUUAUCUUCCAU	243	4357	ACACCUUUUAUCUUCCAU	243	4379	CAUGGAAAGAUAAAAGGUU	670
4375	GGGAGCCAGCUGCUUUUUG	244	4375	GGGAGCCAGCUGCUUUUUG	244	4397	CAAAAAGCAGCUGGUCCCC	671
4393	GUGAUUUUUUAAGUGC	245	4393	GUGAUUUUUUAAGUGC	245	4415	GCACUUAJUAAAUAUCAC	672
4411	CUUUUUUUUUUGACUAAC	246	4411	CUUUUUUUUUUGACUAAC	246	4433	GUUAGGUCAAAAUAAG	673

4429	CAAGAAUGUAACUCCAGAU	247	4429	CAAGAAUGUAACUCCAGAU	247	4451	AUCUGGAGGUACAUUCUUG	674
4447	UAGAGAAAUGUGACAAGU	248	4447	UAGAGAAAUGUGACAAGU	248	4469	ACUUGUCACUAAUUCUCUA	675
4465	UGAAGAACACUACUGCUAA	249	4465	UGAAGAACACUACUGCUAA	249	4487	UUAGCAGUAGUGUUCUUA	676
4483	AAUCCUCAUAGUACUCAGU	250	4483	AAUCCUCAUAGUACUCAGU	250	4505	ACUGAGUAACAUAGGGAUU	677
4501	UGUUAGAGAAAUCUUCU	251	4501	UGUUAGAGAAAUCUUCU	251	4523	AGGAAGGAAUUCUUAACA	678
4519	UAAACCCAAUGACUCCU	252	4519	UAAACCCAAUGACUCCU	252	4541	AGGGAAAGUACAUUGGUUUA	679
4537	UGCUCCAACCCCCGCCACC	253	4537	UGCUCCAACCCCCGCCACC	253	4559	GGGGGGGGGUUGGGAGCA	680
4555	CUCAGGGCACGAGGACCA	254	4555	CUCAGGGCACGAGGACCA	254	4577	UGGUCCUGGGGCCCCUGAG	681
4573	AGUUUAGAUUUGGGAGCUGC	255	4573	AGUUUAGAUUUGGGAGCUGC	255	4595	GCAGCUCCUCAUCAAAACU	682
4591	CACUJAUCAACCCAAUJCAU	256	4591	CACUJAUCAACCCAAUJCAU	256	4613	AUGCAUUGGGUGALICAGUG	683
4609	UCACGUACCCCCACUGGGCC	257	4609	UCACGUACCCCCACUGGGCC	257	4631	GGCCCAGUUGGGGUACGGUGA	684
4627	CAGCCCCUGCAGCCCCAAC	258	4627	CAGCCCCUGCAGCCCCAAC	258	4649	GUUUUGGGUGGGGGCUG	685
4645	CCCAGGGCAACAAAGCCGU	259	4645	CCCAGGGCAACAAAGCCGU	259	4667	ACGGGCUUJGUUGGCCUGGG	686
4663	UAGGCCCAAGGGGAUCACU	260	4663	UAGGCCCAAGGGGAUCACU	260	4685	AGUGAUCCCCUGGGGUCAA	687
4681	UGGCUGGCCUGAGCAACAU	261	4681	UGGCUGGCCUGAGCAACAU	261	4703	AUGUUGUCAGGCCAGCCA	688
4699	UCUCGGGGAGGUCCUCAUGCA	262	4699	UCUCGGGGAGGUCCUCAUGCA	262	4721	UGCUAGAGGACUCCCGAGA	689
4717	AGGCCUAAGACAUGUGAGG	263	4717	AGGCCUAAGACAUGUGAGG	263	4739	CCUCACAUUGUCUUAAGGCCU	690
4735	GAGGAAAAGGAAAAAAAGC	264	4735	GAGGAAAAGGAAAAAAAGC	264	4757	GCUUUUUUUCUUUUCUCUC	691
4753	CAAAAAGCAAGGGAGAAAA	265	4753	CAAAAAGCAAGGGAGAAAA	265	4775	UUUUUCUCCCCGUUUUUUG	692
4771	AGAGAAAACGGGAGAAGGC	266	4771	AGAGAAAACGGGAGAAGGC	266	4793	GCCUUUCUCCCCGUUUCUCU	693
4789	CAUAGAAAAGAAUJUJAGA	267	4789	CAUAGAAAAGAAUJUJAGA	267	4811	UCUCAAUUUUUCUUCUAG	694
4807	ACGCACCAUGGGCACGG	268	4807	ACGCACCAUGGGCACGG	268	4829	CCGUGCCCCACAUUGUGCGU	695
4825	GAGGGGGACGGGGUCAGC	269	4825	GAGGGGGACGGGGUCAGC	269	4847	GCUGAGGCCGUCCCCCUC	696
4843	CAAUGCCAUUUCAGGGCU	270	4843	CAAUGCCAUUUCAGGGCU	270	4865	AGCCACAUAAAUGGCAUUG	697
4861	UCCCAAGCUCUGACCUUC	271	4861	UCCCAAGCUCUGACCUUC	271	4883	GAAGGGGUCAAGGCGUGGAA	698
4879	CUACAUUUGGGGCCAGC	272	4879	CUACAUUUGGGGCCAGC	272	4901	GCUGGGCCCUCUAAAGUAG	699
4897	CCAGGAGCAGAUGGACAGC	273	4897	CCAGGAGCAGAUGGACAGC	273	4919	GCUGUCCAUUCGUCCUGG	700
4915	CGAUGAGGGGACAUUUUC	274	4915	CGAUGAGGGGACAUUUUC	274	4937	AGAAAAUGUCCCCCUCAUCG	701
4933	UGGAUUCUGGGAGGCAAGA	275	4933	UGGAUUCUGGGAGGCAAGA	275	4955	UCUUGCCUCCCCAGAAUCCA	702
4951	AAAAGGACAAAUAUCUUUU	276	4951	AAAAGGACAAAUAUCUUUU	276	4973	AAAAGAUUAUJGUUCUUUU	703
4969	UUUGGAACUAAAAGCAAAU	277	4969	UUUGGAACUAAAAGCAAAU	277	4991	AUUUGCUUAGUUCUAAA	704
4987	UUUAGACCUUACCUAUGG	278	4987	UUUAGACCUUACCUAUGG	278	5009	CCAUAGGUAAAAGGUUAAA	705
5005	GAAGUGGUUCUAGGUCCAU	279	5005	GAAGUGGUUCUAGGUCCAU	279	5027	AUGGACAUAGAACACUUC	706
5023	UUCUCAUUCGGGCAUGUU	280	5023	UUCUCAUUCGGGCAUGUU	280	5045	AAACAUUGCACGGAAUAGAGA	707
5041	UUUGAUUJGUAGCACUGAG	281	5041	UUUGAUUJGUAGCACUGAG	281	5063	CUCAGGUUCUACAAUCAAA	708
5059	GGGGGGCACUCAACUCUGA	282	5059	GGGGGGCACUCAACUCUGA	282	5081	UCAGAGUUGAGGGCCACCC	709

5077	AGCCCAUACUUUGGUCC	283	5077	AGCCCAUACUUUGGUCC	283	5099	GGAGCCAAAAGUAUGGGCU	710
5095	CUCUAGUAAGGACUGA	284	5095	CUCUAGUAAGGACUGA	284	5117	UCAGUGGCAUCUUAUGAG	711
5113	AAAACUUAAGCCAGGUAG	285	5113	AAAACUUAAGCCAGGUAG	285	5135	CUAACUCUGGGCUAAGUUUU	712
5131	GGUUGUCUCCAGGCCAUGA	286	5131	GGUUGUCUCCAGGCCAUGA	286	5153	UCAUGGCCUGGAGACAAAC	713
5149	AUGGCCUUAACACUGAAAAU	287	5149	AUGGCCUUAACACUGAAAAU	287	5171	AUUUCAGUGUAAGGCCAU	714
5167	UGUCACAUUCUAAUUGGG	288	5167	UGUCACAUUCUAAUUGGG	288	5189	CCCAAAAAGAAUUGUGACA	715
5185	GUAUAAAUAUAUAGUCCAG	289	5185	GUAUAAAUAUAUAGUCCAG	289	5207	CUGGACUUAUAUUAUAC	716
5203	GACACUUAACUAAUUCU	290	5203	GACACUUAACUAAUUCU	290	5225	AGAAAUAUGGUUAAGUGUC	717
5221	UJGGUUAUJAUCUGUUUJG	291	5221	UJGGUUAUJAUCUGUUUJG	291	5243	CAAAACAGAAAUAUACCAA	718
5239	GCACAGUUAJGUUGGAAAAG	292	5239	GCACAGUUAJGUUGGAAAAG	292	5261	CUUUCACAAACUACUGUGC	719
5257	GAAAGCUGAGAAAGAUGAA	293	5257	GAAAGCUGAGAAAGAUGAA	293	5279	UUCAUUCUUCUCAGCUUUC	720
5275	AAAUGCAGGUCCUGAGGAGA	294	5275	AAAUGCAGGUCCUGAGGAGA	294	5297	UCUCCUCAGGACUGCAUUU	721
5293	AGUUUUCUCCAUACAAA	295	5293	AGUUUUCUCCAUACAAA	295	5315	UUUUGAUAGGGAAAACU	722
5311	ACGAGGGCUGAUGGAGGA	296	5311	ACGAGGGCUGAUGGAGGA	296	5333	UUCUCCAUACUGCCUCGU	723
5329	AAAAGGUCAUAAGGUCAA	297	5329	AAAAGGUCAUAAGGUCAA	297	5351	UUGACCUUAUUGACCUUUU	724
5347	AGGGAAAGCCCCGUUCUCA	298	5347	AGGGAAAGCCCCGUUCUCA	298	5369	UAGAGACGGGGGUUCUCCU	725
5365	AUACCAACCAAAACCAAUUC	299	5365	AUACCAACCAAAACCAAUUC	299	5387	GAUUUGGUUUUGGUUGUAU	726
5383	CACCAACACAGUUGGGACC	300	5383	CACCAACACAGUUGGGACC	300	5405	GGUCCCAACUGUGIUGGUG	727
5401	CCAAAAACACAGGAAGUCAG	301	5401	CCAAAAACACAGGAAGUCAG	301	5423	CUGACUUCUGUGUUUUGG	728
5419	GUCACGUUUCCUUCUCAUU	302	5419	GUCACGUUUCCUUCUCAUU	302	5441	AAUGAAAGGAAAACUGUGAC	729
5437	UUAAAUGGGAUUCCACAUU	303	5437	UUAAAUGGGAUUCCACAUU	303	5459	AUAGUGGAAUCCCAUAAA	730
5455	UCUCACACAUACUGAAAG	304	5455	UCUCACACAUACUGAAAG	304	5477	CUUUCAGAUAGUGUGAGA	731
5473	GGAUUGGGAAGGCAUUAG	305	5473	GGAUUGGGAAGGCAUUAG	305	5495	CUAAUGCUCUUCACACAUCC	732
5491	GCUGGGCGCAUAUUAAGCAC	306	5491	GCUGGGCGCAUAUUAAGCAC	306	5513	GUGCUUUUAUGGCCAGC	733
5509	CUUAAAGGUCCUUGGUAA	307	5509	CUUAAAGGUCCUUGGUAA	307	5531	UUACUCAAGGAGGUAAAAG	734
5527	AAAAGGUUGGUAGGUUUUU	308	5527	AAAAGGUUGGUAGGUUUUU	308	5549	AAAUAUACAUACCACUUUU	735
5545	UAUGCAAGGUUUUCUCCA	309	5545	UAUGCAAGGUUUUCUCCA	309	5567	UGGAGAAAUAUCCUUGCAUA	736
5563	AGUUGGGACUACGGAUUU	310	5563	AGUUGGGACUACGGAUUU	310	5585	AUAUACCUUGAGUCCCAACU	737
5581	UAGUUAUAGGACCAUCACU	311	5581	UAGUUAUAGGACCAUCACU	311	5603	AGUGAUGGGCUCAUUAACUA	738
5599	UAGAAGAAAAGCCCAUUU	312	5599	UAGAAGAAAAGCCCAUUU	312	5621	AAAAGGGGUUUUCUUCUA	739
5617	UCAACUGCUUUGAAACUUG	313	5617	UCAACUGCUUUGAAACUUG	313	5639	CAAGUUUCAAGCAGUUGA	740
5635	GCCUGGGGUGCUGAGCAUGA	314	5635	GCCUGGGGUGCUGAGCAUGA	314	5657	UCAUGCUCAGACCCAGGC	741
5653	AUGGGAAUAGGGAGACAGG	315	5653	AUGGGAAUAGGGAGACAGG	315	5675	CCUGUGCUCCUAUUCCAU	742
5671	GGUAGGAAAGGGGCCUAC	316	5671	GGUAGGAAAGGGGCCUAC	316	5693	GUAGGGGCCUUUCUAC	743
5689	CUCUUCAGGGGUCAAAAGAU	317	5689	CUCUUCAGGGGUCAAAAGAU	317	5711	AUCUUUAGACCCUGAAGAG	744
5707	UCAAGUGGGCCUUGGAUCG	318	5707	UCAAGUGGGCCUUGGAUCG	318	5729	CGAUCCAAGGCCACUUGA	745

5725	GCUAAGCUGGCCUCGUUUUG	319	5725	GCUAAGCUGGCCUCGUUUUG	319	5747	CAAACAGAGCCAGGUUAGC	746
5743	GAUGCUAUAUUAUGCAAGU	320	5743	GAUGCUAUAUUAUGCAGUU	320	5765	AACUUGCAUAAAUGCAUC	747
5761	UAGGGGCUUAUGGUAAAUGG	321	5761	UAGGGGCUUAUGGUAAAUGG	321	5783	CCUAAAUAUACAUAGACCCUA	748
5779	GAUGCGCCUACUCUUCAGG	322	5779	GAUGCGCCUACUCUUCAGG	322	5801	CCUGAAGAGGUAGGCCAUC	749
5797	GGUCUAAAAGAUCAAGGGG	323	5797	GGUCUAAAAGAUCAAGGGG	323	5819	CCCACUIGAUUUAUAGACC	750
5815	GCCUUGGAUCGCCUAAGCG	324	5815	GCCUUGGAUCGCCUAAGCG	324	5837	CAGCUUAGGAUCCAAGGGC	751
5833	GGCUCUGUUUGAUGGUUU	325	5833	GGCUCUGUUUGAUGGUUU	325	5855	AAUAGCAUAAAAGAGGCC	752
5851	UU AUGCAAGUAGGGGCUA	326	5851	UU AUGCAAGUAGGGGCUA	326	5873	UAGACCUAACUUGCAUAA	753
5869	AUGUAUUUAGGAUCUGC	327	5869	AUGUAUUUAGGAUCUGC	327	5891	GCAGACAUCCUAAAUCACAU	754
5887	CACCUUUCUGCCAGCUA	328	5887	CACCUUUCUGCCAGCUA	328	5909	UGACUGGGCUGGAGAAGGGUG	755
5905	AGAAGCUGGAGGCAACA	329	5905	AGAAGCUGGAGGCAACA	329	5927	UGUUGCCUCUCCAGCUUCU	756
5923	AGUGGAUUGCUCUUCUUG	330	5923	AGUGGAUUGCUCUUCUUG	330	5945	CAAGAAGCAGCAUCCACU	757
5941	GGGGAGAAGGUAAUGCUC	331	5941	GGGGAGAAGGUAAUGCUC	331	5963	GAAGCAUACUCUUCUCCCC	758
5959	CCUUUUUAUCCAUUUUU	332	5959	CCUUUUUAUCCAUUUUU	332	5981	AAAUAUACAUUGGUAAAAGG	759
5977	UAACUGUAGAACCUAGCU	333	5977	UAACUGUAGAACCUAGCU	333	5999	AGCUCAGGUUUCUACAGUUA	760
5995	UCUAAGUAACCGGAAAGU	334	5995	UCUAAGUAACCGGAAAGU	334	6017	CAUUCUICGGGUUACUJAGA	761
6013	GUAUUGCUCUCGUUCCUUAUG	335	6013	GUAUUGCUCUCGUUCCUUAUG	335	6035	CAUAGAACAGAGGCAUAC	762
6031	GUGCCACAUCCUUGUUAA	336	6031	GUGCCACAUCCUUGUUAA	336	6053	UAAAACAGGAUGUGGGCAC	763
6049	AAGGCUCUCUGUAGGAAGA	337	6049	AAGGCUCUCUGUAGGAAGA	337	6071	UCUUCAUACAGAGGCCUU	764
6067	AGAUGGGACCGUACUCAGC	338	6067	AGAUGGGACCGUACUCAGC	338	6089	GCUGAUGACGGGUCCAUCAUC	765
6085	CACAUUCUCCUAGUGAGCCU	339	6085	CACAUUCUCCUAGUGAGCCU	339	6107	AGGCUCACUAGGGAAUGUG	766
6103	UACUGGCCUCCUGGAGCGG	340	6103	UACUGGCCUCCUGGAGCGG	340	6125	CCGCUGCCAGGAGCCAGUA	767
6121	GCUUUUUGGGAAAGACUCAC	341	6121	GCUUUUUGGGAAAGACUCAC	341	6143	GUGAGUCUUCUCCAGGAA	768
6139	CUAGCCAGAAAGAGGGAGU	342	6139	CUAGCCAGAAAGAGGGAGU	342	6161	ACUCCUCUUCUCCUGGUAG	769
6157	UGGGACAGGUCCUCUCCACC	343	6157	UGGGACAGGUCCUCUCCACC	343	6179	GGUGGAGAGGGACUGUCCCA	770
6175	CAAGAUCAAAUCCAAAACA	344	6175	CAAGAUCAAAUCCAAAACA	344	6197	UGUUUUGAUUUUAGAUUCUUG	771
6193	AAAAGCAGGGCUAGGCCAG	345	6193	AAAAGCAGGGCUAGGCCAG	345	6215	CUGGCUCUAGGCCUGGUUUU	772
6211	GAAGAGAGGACAAAUUU	346	6211	GAAGAGAGGACAAAUUU	346	6233	AAAGAUUUGGUCCUCUUCUUC	773
6229	UGUUGUUCUCUUCUUCUUC	347	6229	UGUUGUUCUCUUCUUCUUC	347	6251	GUAAAAGAGGGAAACAACA	774
6247	CACAUACGCAAACCCACUG	348	6247	CACAUACGCAAACCCACUG	348	6269	CAGGGGUUUGGGUAGUGUG	775
6265	GUGACAGCUGGCAAUUUUA	349	6265	GUGACAGCUGGCAAUUUUA	349	6287	UAAAUGGCCAGCUGUCAC	776
6283	AUAAAUCAGGUAAUCUGGA	350	6283	AUAAAUCAGGUAAUCUGGA	350	6305	UJCCAGLUACCLGUAUJAU	777
6301	AGGGGGUAAAACUCAGAAA	351	6301	AGGGGGUAAAACUCAGAAA	351	6323	UUUCUGGUUUUACCUCCU	778
6319	AAAAGAAGACCUAGCUAA	352	6319	AAAAGAAGACCUAGCUAA	352	6341	UUGACUGAGGGUCUICUUUU	779
6337	AUUCUCUACUUUUUUUUU	353	6337	AUUCUCUACUUUUUUUUU	353	6359	AAAAAAAAGUAGAGAAU	780
6355	UUUUUUUCCAAAUCAGAU	354	6355	UUUUUUUCCAAAUCAGAU	354	6377	UAUCUGAUUUUGGAAAAAA	781

6373	AAUAGCCAGCAAAUAGUG	355	6373	AAUAGCCAGCAAAUAGUG	355	6395	CAUCAUUUGCUGGGCUAUU	782
6391	GAUAAACAAUAAAACCUCU	356	6391	GAUAAACAAUAAAACCUCU	356	6413	UAAGGUUUUUUUUUUAUC	783
6409	AGCUGUUCAUGUCUGUAU	357	6409	AGCUGUUCAUGUCUGUAU	357	6431	AAUCAAGACAAUGAAACGGCU	784
6427	UUCAAUAAAUAUUCUUAU	358	6427	UUCAAUAAAUAUUCUUAU	358	6449	UUAAGAAUUAUUAUUGAA	785
6445	AUCAUUAGAGACCAAAU	359	6445	AUCAUUAGAGACCAAAU	359	6467	AUUAUGGUUCUCUUAUUGAU	786
6463	UAAAUAUCUCCUUUCAAGA	360	6463	UAAAUAUCUCCUUUCAAGA	360	6485	UCUUGAAAAGGAGUAUUA	787
6481	AGAAAAAGCAAAACCACUAG	361	6481	AGAAAAAGCAAAACCACUAG	361	6503	CUAAUGGUUUUGCUUUUCU	788
6499	GAUUGUUAUCAGCUCCU	362	6499	GAUUGUUAUCAGCUCCU	362	6521	AGGAGCUGAGUAACAAUUC	789
6517	UUCAAAUCAGGUUGUAG	363	6517	UUCAAAUCAGGUUGUAG	363	6539	CUACAAAACCUGAGUUUGAA	790
6535	GCAUACAUAGAGCUCAUCA	364	6535	GCAUACAUAGAGCUCAUCA	364	6557	UGGAUGGACUCAUAGUAUGC	791
6553	AUCAGCUAAAGAAUGGUUC	365	6553	AUCAGCUAAAGAAUGGUUC	365	6575	GAACCAUUCUUGACUGAU	792
6571	CCAUCUGGAGCUUAAUGU	366	6571	CCAUCUGGAGCUUAAUGU	366	6593	ACAUUAAGACUCCAGAUGG	793
6589	UAGAAAAGAAAAAUGGAGAC	367	6589	UAGAAAAGAAAAAUGGAGAC	367	6611	GUCUCCAUUUUCUUCUUCUA	794
6607	CUUGUAAAUAUGAGCUAGU	368	6607	CUUGUAAAUAUGAGCUAGU	368	6629	ACUAGCUCAUUUUACAAG	795
6625	UUACAAAUGGCUUUCUCAU	369	6625	UUACAAAUGGCUUUCUCAU	369	6647	AUGAACAAAGCACUUUGUAA	796
6643	UUAAAUAUAGCACUGAAAAU	370	6643	UUAAAUAUAGCACUGAAAAU	370	6665	AUUUUCAUGGCUAUUUUUA	797
6661	UUGAAAACAUAGAAUUAUCG	371	6661	UUGAAAACAUAGAAUUAUCG	371	6683	CAGUAAAUCAUAGUUUCUAA	798
6679	GAUAAAUAUCCAAUCAUUU	372	6679	GAUAAAUAUCCAAUCAUUU	372	6701	AAAUGAUUGGAAUUAUC	799
6697	UGCCAUUUUAUGACAAAAAU	373	6697	UGCCAUUUUAUGACAAAAAU	373	6719	AUUUUUGCUAUAAAUGGCA	800
6715	UGGUUGGCACUAACAAAGA	374	6715	UGGUUGGCACUAACAAAGA	374	6737	UCUUUUGUAGUGCCAAACCA	801
6733	AACGAGGACACUUCUUCAG	375	6733	AACGAGGACACUUCUUCAG	375	6755	CUGAAAAGGAAGUGCUUCGUU	802
6751	GAGUUUCUGAGAUAAUGUA	376	6751	GAGUUUCUGAGAUAAUGUA	376	6773	UACAUUAUCUCAGAAACUC	803
6769	ACGUGGAACAGUCUGGGUG	377	6769	ACGUGGAACAGUCUGGGUG	377	6791	CACCCAGACUGUUCACAGU	804
6787	GGAAUAGGGCGUGAAACAU	378	6787	GGAAUAGGGCGUGAAACAU	378	6809	AUGGUUUCAUGGCCCAUUC	805
6805	UGUGGAAGGUGUGUUCUUG	379	6805	UGUGGAAGGUGUGUUCUUG	379	6827	CAAGACACAGACUUGGCACA	806
6823	GUCAGGUCCAAGAUGGACA	380	6823	GUCAGGUCCAAGAUGGACA	380	6845	UGUCACUUCUUGGACUGAC	807
6841	ACCGAGAUGUAAAUUUAG	381	6841	ACCGAGAUGUAAAUUUAG	381	6863	CUAAAAAUAAACAUUCUCGGU	808
6859	GGGACCCGUGCCUUGUUUC	382	6859	GGGACCCGUGCCUUGUUUC	382	6881	GAACACAGGGCACGGGUCCC	809
6877	CCUAGCCCACAAAGAUGCA	383	6877	CCUAGCCCACAAAGAUGCA	383	6899	UGCAUUUCUUGGGCUUAGG	810
6895	AAACAUCAAAACAGAUACUC	384	6895	AAACAUCAAAACAGAUACUC	384	6917	GAGUAUCUGUUUGAUUGUUU	811
6913	CGCUAGCCUCAUUAAAUU	385	6913	CGCUAGCCUCAUUAAAUU	385	6935	AUUUUAAAUGGGCUUAGCG	812
6931	UGAUAAAAGGAGGAUGCA	386	6931	UGAUAAAAGGAGGAUGCA	386	6953	UGCACUCUCUUUUAUCA	813
6949	AUCUUUGGCCGACAGUGGU	387	6949	AUCUUUGGCCGACAGUGGU	387	6971	ACACACUGGGCCAAAGAU	814
6967	UGUAACUGUGUGUGUGUGU	388	6967	UGUAACUGUGUGUGUGUGU	388	6989	ACACACACACACAGGUACAC	815
6985	UGUGUGUGUGUGUGUGUGU	389	6985	UGUGUGUGUGUGUGUGUGU	389	7007	ACACACACACACACACACACA	816
7003	UGUGUGUGUGUGUGUGUGU	390	7003	UGUGUGUGUGUGUGUGUGU	390	7025	CCACACCCACACACACACACA	817

7021	GGUGUAUGUGUUUUGUG	391	7021	GGUGUAUGUGUUUUGUG	391	7043	CACAAACACACAUACACC	818
7039	GCAUAACUAAAAGAAAA	392	7039	GCAUAACUAAAAGAAAA	392	7061	UUUCUUAUAGUUAUGC	819
7057	ACUGGAAUUUAAAAGUAC	393	7057	ACUGGAAUUUAAAAGUAC	393	7079	GUACUUUUAAAUCAGU	820
7075	CUUUUAACAAACCAAGAA	394	7075	CUUUUAACAAACCAAGAA	394	7097	UUCUUGGUUUUAGAAAAG	821
7093	AUAUAUGCUACAGAUAAA	395	7093	AUAUAUGCUACAGAUAAA	395	7115	UUAUAUCGUAGCAUAAA	822
7111	AGACAGACAUUGGUJGGUC	396	7111	AGACAGACAUUGGUJGGUC	396	7133	GACCAACCAUGUCUGUCU	823
7129	CCUUAUUCUAGCUAUGA	397	7129	CCUUAUUCUAGCUAUGA	397	7151	UCAUGACUAGAAAUAUAGG	824
7147	AUGAAUGUAAAUGUAC	398	7147	AUGAAUGUAAAUGUAC	398	7169	GUAAACAAAUAUCUUCAU	825
7165	CCAUUCUCAUAAAUAUAC	399	7165	CCAUUCUCAUAAAUAUAC	399	7187	GUAAUAAAUAUGAAAGAUGG	826
7183	CUUUAAAUAUUCUJUAAA	400	7183	CUUUAAAUAUUCUJUAAA	400	7205	AUUAAGAAAUAUJUAAAAG	827
7201	UUGGGAUUUGUAAUCGUAC	401	7201	UUGGGAUUUGUAAUCGUAC	401	7223	GUACGAAUACAAAUCCAA	828
7219	CCAAUCUUAAAUGUAAACU	402	7219	CCAAUCUUAAAUGUAAACU	402	7241	AGUUUUAUCAAAUAUGUUGG	829
7237	UUGGCAACUGCUUUUAUGU	403	7237	UUGGCAACUGCUUUUAUGU	403	7259	ACAUAAAAGCAGUUCCCAA	830
7255	UUCUGUCUCCUJUCCAUAAA	404	7255	UUCUGUCUCCUJUCCAUAAA	404	7277	UUUAGGAGGGAGACAGAA	831
7273	AUUUUUCAAAAACUAAA	405	7273	AUUUUUCAAAAACUAAA	405	7295	AAUUAUAAUUUUAAAUAU	832
7291	UCAACAAAAGCUCU	406	7291	UCAACAAAAGCUCU	406	7313	AGAGCUUUUUCUUGUJUGA	833
7309	UUUUUUUUCUAAAUAAAA	407	7309	UUUUUUUUCUAAAUAAAA	407	7331	UUUUUUUJAGGAAAAAAA	834
7327	ACUCAAAUUAUCCUJGUU	408	7327	ACUCAAAUUAUCCUJGUU	408	7349	AACAAGGAUAAAUGAGU	835
7345	UAGAGCAGAGAAAAAAA	409	7345	UAGAGCAGAGAAAAAAA	409	7367	UAAAUUUUCUCUGUCUUA	836
7363	AAGAAAAACUUUGAAUAGG	410	7363	AAGAAAAACUUUGAAUAGG	410	7385	CCAUUUCAAGUUUUUCUU	837
7381	GUUCUCAAAAAAUUGCUAAA	411	7381	GUUCUCAAAAAAUUGCUAAA	411	7403	UUJAGCAUJUJJUGAGAC	838
7399	AUAUJJUCAAGGAAAACU	412	7399	AUAUJJUCAAGGAAAACU	412	7421	AGUUUUCCAUUUGAAAAAU	839
7417	UAAAUGUJAGUUUAGCUGA	413	7417	UAAAUGUJAGUUUAGCUGA	413	7439	UCAGCUAAAACUAAUUA	840
7435	AUUGUAUGGGUUUUCGAA	414	7435	AUUGUAUGGGUUUUCGAA	414	7457	UUCGAAAACCCCACUACAAU	841
7453	ACUUUUCACUUUUUUUJUG	415	7453	ACUUUUCACUUUUUUUJUG	415	7475	CAAACAAAAGUGAAAGGU	842
7471	GUUUUACUAAAUCACAC	416	7471	GUUUUACUAAAUCACAC	416	7493	GUUGGAAAAGGUAAAAC	843
7489	CUGUGAAAAGCCAAA	417	7489	CUGUGAAAAGCCAAA	417	7511	UUAUUUGCCAAAUCACAG	844
7507	AUUCUGGUCAAGAAAUG	418	7507	AUUCUGGUCAAGAAAUG	418	7529	CAUUUUCAGGACAGGAAU	845
7525	GCAAAUUAUCCAGGUAGA	419	7525	GCAAAUUAUCCAGGUAGA	419	7547	UCUACACUGGUAAAUGC	846
7543	AUAAUJJUGACCAACCC	420	7543	AUAAUJJUGACCAACCC	420	7565	GGGUGAUJGGGUAAAUAU	847
7561	CUAUGGAAUJGGCUJGUU	421	7561	CUAUGGAAUJGGCUJGUU	421	7583	AAUAGCCAAAUCAUAG	848
7579	UUUGCCUUAAAAGCAAA	422	7579	UUUGCCUUAAAAGCAAA	422	7601	UUUGCCUUAAAAGCAAA	849
7597	AUUCAUUUCAGCCUGAAUG	423	7597	AUUCAUUUCAGCCUGAAUG	423	7619	CAUUCAGGCUGAAAUGAAU	850
7615	GUCUGCCUAAAUCUCU	424	7615	GUCUGCCUAAAUCUCU	424	7637	AGAGAAUAAAUGGCAGAC	851
7633	UGCUCUJGUAAAUCUCU	425	7633	UGCUCUJGUAAAUCUCU	425	7655	AAGGAGAAUAAAAGAGCA	852
7651	UUGAACCCGUAAAACAUC	426	7651	UUGAACCCGUAAAACAUC	426	7673	GAUGUUUUAAAAGGUUCAA	853

7662	AAAACAUCUGUGGCACUC	427	7662	AAAACAUCUGUGGCACUC	427	7684	GAGUGGCACAGGAUGUUU	854
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VEGFR2 gi|11321596 refNM_002253.1

Pos	Target Sequence	Seq ID	UpPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1	ACUGAGUCGGGGACCCCG	855	1	ACUGAGUCGGGGACCCCG	855	23	CGGGGUCCCCGGACUCAGU	1179
19	GGGAGAGGGGUAGUGUGU	856	19	GGGAGAGGGGUAGUGUGU	856	41	ACACACUGACGGCUUCUCC	1180
37	UGGUUCGCGUUCGUUCUCU	857	37	UGGUUCGCGUUCGUUCUCU	857	59	AGAGGAAACGAGCGACCA	1181
55	UGCCUGGCCGGCAUCAC	858	55	UGCCUGGCCGGCAUCAC	858	77	GUGAUGCCCCGGCGAGGCA	1182
73	CUUGCCGCCGGAGAAAGU	859	73	CUUGCCGCCGGAGAAAGU	859	95	ACUUUCUGGGCGCGCAAG	1183
91	UCCGUUCUGGCAGCCUGAU	860	91	UCCGUUCUGGCAGCCUGAU	860	113	AUCCAGGUUCGCCAGACGGA	1184
109	UAUCCUCUCUACCGGCAC	861	109	UAUCCUCUCUACCGGCAC	861	131	GUGCCGGGUAGGAGGGAUA	1185
127	CCCGCAGAGGCCCUUCGAG	862	127	CCCGCAGAGGCCCUUCGAG	862	149	CUGCAGGGGGCUCUGCGGG	1186
145	GCCGCCGGUCGGGCCGG	863	145	GCCGCCGGUCGGGCCGG	863	167	CGGGGCCCGACCGGGGGC	1187
163	GCCUCCCCUAGCCUGUGCG	864	163	GCCUCCCCUAGCCUGUGCG	864	185	CGCACAGGGCUAGGGAGCC	1188
181	GCUCAACUGGUCCUGGGCUG	865	181	GCUCAACUGGUCCUGGGCUG	865	203	CAGCGCAGGACAGUUGAGC	1189
199	GCGGGGUGGCCGGAGUUC	866	199	GCGGGGUGGCCGGAGUUC	866	221	GGAACUCUGGGCACCCGGC	1190
217	CACCUCCGGGCCCUUCU	867	217	CACCUCCGGGCCCUUCU	867	239	AGAAGGGGGCGGGAGGGUG	1191
235	UCUAGACAGGGCUGGGAG	868	235	UCUAGACAGGGCUGGGAG	868	257	CUCCCAGGGCCUGGUAGA	1192
253	GAAAGAACCGGUCCCGAG	869	253	GAAAGAACCGGUCCCGAG	869	275	CUCGGGAGGGGUUCUUUC	1193
271	GUUCUGGGCAUUCGCCCG	870	271	GUUCUGGGCAUUCGCCCG	870	293	CGGGCAAAAGGCCAGAAC	1194
289	GGCUUCGAGGUGGAGGAUGC	871	289	GGCUUCGAGGUGGAGGAUGC	871	311	GCAUCCUGCACCUUGAGCC	1195
307	CAAGAGCAAGGGUGGUGG	872	307	CAAGAGCAAGGGUGGUGG	872	329	CCAGCAGCACCUUGGUCUG	1196
325	GCCGUGGCCUGGGCUCU	873	325	GCCGUGGCCUGGGCUCU	873	347	AGAGCCACAGGGCAGGGC	1197
343	UGCGUGGGAGACCCGGCCG	874	343	UGCGUGGGAGACCCGGCCG	874	365	CGGGCGGGGUUCUCCACGCA	1198
361	GCCUCUJGGGUUJGCUA	875	361	GCCUCUJGGGUUJGCUA	875	383	UAGGCAAACCCACAGAGGC	1199
379	AGUGUUCUCUUGAUCUGC	876	379	AGUGUUCUCUUGAUCUGC	876	401	GCAGAUCAAGAGAAACACU	1200
397	CCCAGGGCUAGCAUAAA	877	397	CCCAGGGCUAGCAUAAA	877	419	UUJGUUAUGCUGGCCUGGG	1201
415	AAAGACAUACUAAUUA	878	415	AAAGACAUACUAAUUA	878	437	UAUUGUUAAGUAGUCUU	1202
433	AAGGCUAAUACAAUCUUC	879	433	AAGGCUAAUACAAUCUUC	879	455	GAAGAGUJGUUAUAGCCUU	1203
451	CAAAUUAUCUJUGCAGGGAC	880	451	CAAAUUAUCUJUGCAGGGAC	880	473	GUCCCCUJGCAAGUAUUUUG	1204
469	CAAGGGGACUUGGACUGGC	881	469	CAAGGGGACUUGGACUGGC	881	491	GCCAGGUCCAAGUCCUCUG	1205
487	CUUUGGCCAAUAAUCAGA	882	487	CUUUGGCCAAUAAUCAGA	882	509	UCUGAUUAUUGGGCCAAAG	1206

505	AGUGGCAGUGAGCAAAGGG	883	505	AGUGGGAGUGAGCAAAGGG	883	527	CCCUUUGUCACUGGCCACU	1207
523	GUGGAGGGUGACUGAGUGCA	884	523	GUGGAGGGUGACUGAGUGCA	884	545	UGCACUCUGACCUCCAC	1208
541	AGCGAUGGCCCUUCUGUA	885	541	AGCGAUGGCCCUUCUGUA	885	563	UACAGAAAGGGCCAUCCGU	1209
559	AAGACACUCACAAUCCAA	886	559	AAGACACUCACAAUCCAA	886	581	UUGGAUJUGUGAGUGUCUU	1210
577	AAAGUGAUCGGAAAUGACA	887	577	AAAGUGAUCGGAAAUGACA	887	599	UGUCAUJUCCGAUCACUU	1211
595	ACUGGGAGCCUACAAGUGCU	888	595	ACUGGGAGCCUACAAGUGCU	888	617	AGCACUJUAGGGCCUCCAGU	1212
613	UUCUACCGGGAAACUGACU	889	613	UUCUACCGGGAAACUGACU	889	635	AGUCAGUUJUCCCGGUAGAA	1213
631	UUGGCCUCGGGUCAUUAUG	890	631	UUGGCCUCGGGUCAUUAUG	890	653	CAUAAUIGACCGAGGCCAA	1214
649	GUICUAUGGUICAAAGAUACA	891	649	GUICUAUGGUICAAAGAUACA	891	671	UGUAAUUCUUGAACAUAGAC	1215
667	AGAUUCUCCAUUUAUJGUU	892	667	AGAUUCUCCAUUUAUJGUU	892	689	AAGCAAUAAAUGGAGAUU	1216
685	UCUGUUAGUGGACCAACAUG	893	685	UCUGUUAGUGGACCAACAUG	893	707	CAUGUUGGGUCACUAAACAGA	1217
703	GGAGUCGUGGUACAUUACUG	894	703	GGAGUCGUGGUACAUUACUG	894	725	CAGUAAUGUACACGACUCC	1218
721	GAGAACAAAAAACAAACUG	895	721	GAGAACAAAAAACAAACUG	895	743	CAGUUUUUUUUUGUUCUC	1219
739	GUUGGUAGUCCUGGUUCUG	896	739	GUUGGUAGUCCUGGUUCUG	896	761	CGAGACAUAGGAAUACCCAC	1220
757	GGGUCCAUUUCAAUCUCA	897	757	GGGUCCAUUUCAAUCUCA	897	779	UGAGAUUJUGAAAUGGACCC	1221
775	AACGUGGUACUJUGGGCAA	898	775	AACGUGGUACUJUGGGCAA	898	797	UGGCACAAAGUGACACGUU	1222
793	AGAUACCCAGAAAAAGAGAU	899	793	AGAUACCCAGAAAAAGAGAU	899	815	AUCUCUUUUCUGGGGUAUU	1223
811	UUUGUJUCCUGAUGGUUACA	900	811	UUUGUJUCCUGAUGGUUACA	900	833	UGUUAUCCAUUCAGGAAACAA	1224
829	AGAAUUIUCUGGGACAGCA	901	829	AGAAUUIUCUGGGACAGCA	901	851	UGCUGUCCAGGAAUUCU	1225
847	AAGAAGGGCUUACAUUUC	902	847	AAGAAGGGCUUACAUUUC	902	869	GAUAGUAAAAGGCCUUUCUU	1226
865	CCCGAGCUACAUAGAUAGCU	903	865	CCCGAGCUACAUAGAUAGCU	903	887	AGCUGAUCAUGUAGCUGGG	1227
883	UAUGCUGGCAUGGUUCUUC	904	883	UAUGCUGGCAUGGUUCUUC	904	905	AGAAGACCAUGCCAGCAUA	1228
901	UGUGAACGAAAAAUUAUG	905	901	UGUGAACGAAAAAUUAUG	905	923	CAUAAAUUUUUGCUUCACAA	1229
919	GAUGAAAGGUACCAGUCA	906	919	GAUGAAAGGUACCAGUCA	906	941	UAGACUGGUAAACUUUCAUC	1230
937	AUUAUGUACAUUGUJUGCG	907	937	AUUAUGUACAUUGUJUGCG	907	959	CGACAACUAUGUACAUAAU	1231
955	GUJGUAGGGGUUAUGGAUU	908	955	GUJGUAGGGGUUAUGGAUU	908	977	AAAUCUUAUACCCUACAC	1232
973	UAUGAUGGUUCUGAGUC	909	973	UAUGAUGGUUCUGAGUC	909	995	GACUCAGAACACAUCAUA	1233
991	CCGUUCUCAUGGAAUJGAAC	910	991	CCGUUCUCAUGGAAUJGAAC	910	1013	GUUCAAUUCCAUAGGACGG	1234
1009	CUAUCUGUJUGGAGAAAAGC	911	1009	CUAUCUGUJUGGAGAAAAGC	911	1031	GUUUUCUCCAACAGAUAG	1235
1027	CUUGUCUAAAUGUACAG	912	1027	CUUGUCUAAAUGUACAG	912	1049	CUGUCAUJUUAAGACAAG	1236
1045	GCAAGAACUGAAACUAAAUG	913	1045	GCAAGAACUGAAACUAAAUG	913	1067	CAUJJUAGGUUCAGUUCUUGC	1237
1063	GUUGGGAGUJUGACUJAAUC	914	1063	GUUGGGAGUJUGACUJAAUC	914	1085	AGUUGAAAGGUCAAUCCCCAC	1238
1081	UGGGAAAUACCCUUUCUUC	915	1081	UGGGAAAUACCCUUUCUUC	915	1103	UCGAAAGGUAGGGGUAUUCCA	1239
1099	AAGCAUCAGCAUAAAAGAAC	916	1099	AAGCAUCAGCAUAAAAGAAC	916	1121	GUUCUUAUGCUGAUGGUU	1240
1117	CUJGUAAAACCGAGACCUAA	917	1117	CUJGUAAAACCGAGACCUAA	917	1139	UAGGGUCUCGGGUUJACAG	1241
1135	AAAACCCAGUCUGGGAGUG	918	1135	AAAACCCAGUCUGGGAGUG	918	1157	CACUCCCCAGACUGGGGUUU	1242

1153	GAGAUGAAGAAUUUUUGA	919	1153	GAGAUGAAGAAUUUUUGA	919	1175	UCAAAAAUUUUUCAUCUC	1243
1171	AGCACCUUACUUAUGAUG	920	1171	AGCACCUUACUUAUGAUG	920	1193	CAUCUAGUUAAGGUGCU	1244
1189	GUUGUAACCCGGAGUGACC	921	1189	GUUGUAACCCGGAGUGACC	921	1211	GGUCACUCCGGGUACACC	1245
1207	CAAGGAUUGUACACCUGUG	922	1207	CAAGGAUUGUACACCUGUG	922	1229	CACGGGUUACAAUCCUUG	1246
1225	GCAGCAUCAGCAGGGCUGA	923	1225	GCAGCAUCAGCAGGGCUGA	923	1247	UCAGCCCACUGGAUGUCGC	1247
1243	AUGACCAAGAAACAGCA	924	1243	AUGACCAAGAAACAGCA	924	1265	UGCUGUUCUUCUUGGUCAU	1248
1261	ACAUUUGUCAGGGGUCAUG	925	1261	ACAUUUGUCAGGGGUCAUG	925	1283	CAUGGACCCUGACAAAUGU	1249
1279	AAAAAACCUUUUGGUUCUU	926	1279	AAAAAACCUUUUGGUUCUU	926	1301	AAGCAACAAAAGGUUUUUC	1250
1297	UUUGGAAGUGGCAUGGAAU	927	1297	UUUGGAAGUGGCAUGGAAU	927	1319	AUUCGAUGCCACUCCAAA	1251
1315	UCUCUGGGGAAGGCCACGG	928	1315	UCUCUGGGGAAGGCCACGG	928	1337	CCGGGGGUUCCACAGAGA	1252
1333	GUCCCCGGGGCGGUCAAGAA	929	1333	GUCCCCGGGGCGGUCAAGAA	929	1355	UUCUGACACGCCUCCCCCAC	1253
1351	AUCCCUUGCGAAGGUACCUUG	930	1351	AUCCCUUGCGAAGGUACCUUG	930	1373	CAAGGUACUUCGCAAGGGAU	1254
1369	GUUACCCACCCCCAGAAA	931	1369	GUUACCCACCCCCAGAAA	931	1391	UUCUGGGGGGGGUUAACC	1255
1387	AUAAAUGGUUAUUAAAUG	932	1387	AUAAAUGGUUAUUAAAUG	932	1409	CAUUUUUAUCCAUUUUAU	1256
1405	GGAAUACCCUUGGUCCCA	933	1405	GGAAUACCCUUGGUCCCA	933	1427	UGGACUCAAGGGGUUUUCC	1257
1423	AUCACACAAUUAAAAGCGG	934	1423	AUCACACAAUUAAAAGCGG	934	1445	CCGGUUUUAAUUGUGUGAUU	1258
1441	GGGCAUGGUACUGACGAUUA	935	1441	GGGCAUGGUACUGACGAUUA	935	1463	UAAUCGUACUGUACUGGCC	1259
1459	AUGGAAGUGAGUGAAAGAG	936	1459	AUGGAAGUGAGUGAAAGAG	936	1481	CUCUUUCACUCUCCAU	1260
1477	GACACAGGAAUUACACUG	937	1477	GACACAGGAAUUACACUG	937	1499	CAGGUUAUUCCUGUGUC	1261
1495	GUCAUCCUUAACCCCA	938	1495	GUCAUCCUUAACCCCA	938	1517	UGGGAUUUGGUAGGAUGAC	1262
1513	AUUUCAAAGGAGAAGCAGA	939	1513	AUUUCAAAGGAGAAGCAGA	939	1535	UCUGGUUCUCCUUUJGAAAU	1263
1531	AGCCAUGGGGUUCUCUGG	940	1531	AGCCAUGGGGUUCUCUGG	940	1553	CCAGAGGACCCAUACACAAC	1264
1549	GUUGUGUAUGGUCCACCCC	941	1549	GUUGUGUAUGGUCCACCCC	941	1571	GGGGGGGACAUACACAAC	1265
1567	CAGAUUGGUAGAAUUCUC	942	1567	CAGAUUGGUAGAAUUCUC	942	1589	GAGAUUUUCUCCACCAUCUG	1266
1585	CUAAUCUCUCCUGGUCAU	943	1585	CUAAUCUCUCCUGGUCAU	943	1607	AAUCCACAGGAGGAGUJAG	1267
1603	UCCUACCUAGUACGGACCA	944	1603	UCCUACCUAGUACGGACCA	944	1625	UGGUGCCGUACUGGUAGGA	1268
1621	ACUCAAACCCUGACAUUA	945	1621	ACUCAAACCCUGACAUUA	945	1643	UACAUAGUACGGGUUGAGU	1269
1639	ACGGGUCAUAGCCAUCCUC	946	1639	ACGGGUCAUAGCCAUCCUC	946	1661	GAGAAUUGGUAGACCGGU	1270
1657	CCCCCGCAUCACAUCCACU	947	1657	CCCCCGCAUCACAUCCACU	947	1679	AGUGGAUGUGAUGGGGG	1271
1675	UGGUAUUGGCAUGUUGGAGG	948	1675	UGGUAUUGGCAUGUUGGAGG	948	1697	CCUCCAACUGCCAAUACCCA	1272
1693	GAAGAGUGGCCAACGAGC	949	1693	GAAGAGUGGCCAACGAGC	949	1715	GCUCGUUGGGCACUCUUC	1273
1711	CCCAAGCCAAGGUUCUCAG	950	1711	CCCAAGCCAAGGUUCUCAG	950	1733	CUGAGACAGGUUGGUUGGG	1274
1729	GUAGACAAACCCAUACCCUU	951	1729	GUAGACAAACCCAUACCCUU	951	1751	AAGGGUAUGGGGUUGUCAC	1275
1747	UGUGAAGAAUGGAGAAGUG	952	1747	UGUGAAGAAUGGAGAAGUG	952	1769	CACUUCUCCAUUCUUCACA	1276
1765	GUJGGAGGUUCGGGAG	953	1765	GUJGGAGGUUCGGGAG	953	1787	CUCCCUGGAAGGUCCUCAC	1277
1783	GGAAAAUUUUUUAGGUUA	954	1783	GGAAAAUUUUUUAGGUUA	954	1805	UAAUCUCAUUUUUUUCC	1278

1801	AAUAAAAAUCAAAUUGCUC	955	1801	AAUAAAAAUCAAAUUGCUC	955	1823	GAGCAAAUUGAUUUUAUU	1279
1819	CUAAUUGAAGGAAAAAACAA	956	1819	CUAAUUGAAGGAAAAAACAA	956	1841	UGUUUUUUCCUCAAUUAG	1280
1837	AAAACUGUAAGUACCUUUG	957	1837	AAAACUGUAAGUACCCUUG	957	1859	CAAGGGUACUUAAGUUUU	1281
1855	GUUAUCCAAGGGCAAAUUG	958	1855	GUUAUCCAAGGGCAAAUUG	958	1877	CAUUGCCGUUGAUUAC	1282
1873	GUIGUCAGCUUJGUACAAAU	959	1873	GUIGUCAGCUUJGUACAAAU	959	1895	AUUGUACAAAGCUGACAC	1283
1891	UGUGAAGGGGUCAACAAAG	960	1891	UGUGAAGGGGUCAACAAAG	960	1913	CUUUGUUGACCGCUUJACAA	1284
1909	GUCCGGAGGGAGGAGGG	961	1909	GUCCGGAGGGAGGAGGG	961	1931	CCCCUCUCUCCUCGCCAC	1285
1927	GUGAUCUCUCCUACGUGA	962	1927	GUGAUCUCUCCUACGUGA	962	1949	UCACGUGGAAGGAGAUCAC	1286
1945	ACCAGGGGUCCUGAAUUA	963	1945	ACCAGGGGUCCUGAAUUA	963	1967	UAAUUUCAAGGCCUUGGU	1287
1963	ACUUUGCAACCUACUGC	964	1963	ACUUUGCAACCUACUGC	964	1985	GCAUGUCAGGUUGCAAAAGU	1288
1981	CAGCCCCACUGAGCAGGAGA	965	1981	CAGCCCCACUGAGCAGGAGA	965	2003	UCUCCUGCUCAGUGGGCUG	1289
1999	AGCGGUGCUUJUGGGUGCA	966	1999	AGCGGUGCUUJUGGGUGCA	966	2021	UGCACCAAAAGACACGCCU	1290
2017	ACUGCAGACAGAUCUACGU	967	2017	ACUGCAGACAGAUCUACGU	967	2039	ACGUAGAUCUGUCUGCAGU	1291
2035	UUUGAGAAACCUCACAUUGG	968	2035	UUUGAGAAACCUCACAUUGG	968	2057	ACCAUGUGAGGUUCUAAA	1292
2053	UACAAGCUUJGGCCACAGC	969	2053	UACAAGCUUJGGCCACAGC	969	2075	GCUGUGGGCCAAGCUUGUA	1293
2071	CCUCUGCCAAUCCAUUGGG	970	2071	CCUCUGCCAAUCCAUUGGG	970	2093	CCACAUUGAUJGGCAGAGG	1294
2089	GGAGAGGUUGGCCACACCUG	971	2089	GGAGAGGUUGGCCACACCUG	971	2111	CAGGUGUGGGCAACUCUCC	1295
2107	GUUUGCAAGGAACUJGGAUA	972	2107	GUUUGCAAGGAACUJGGAUA	972	2129	UAUCCAAGGUUCUUGCAAAC	1296
2125	ACUCUJUGGAAAUJGGAUG	973	2125	ACUCUJUGGAAAUJGGAUG	973	2147	CAUUCAUUUCCAAAGAGU	1297
2143	GCCACCAUGUUCCUAAUA	974	2143	GCCACCAUGUUCCUAAUA	974	2165	UAUUAGAGAACAUUGUGGC	1298
2161	AGCACAAUJGACAUUJUGA	975	2161	AGCACAAUJGACAUUJUGA	975	2183	UCAAAAAGUICAUUUUGGU	1299
2179	AUCAUGGGAGCUUAGAAUG	976	2179	AUCAUGGGAGCUUAGAAUG	976	2201	CAUUCUUAAGCUCAUGAU	1300
2197	GCAUCCUJUGCAGGACCAAG	977	2197	GCAUCCUJUGCAGGACCAAG	977	2219	CUUGGUCCUGCAAGGAUGC	1301
2215	GGAGACUAUGUCUGCCUUG	978	2215	GGAGACUAUGUCUGCCUUG	978	2237	CAAGGCAGACAUAGUCUCC	1302
2233	GCUCUAGAGCAGGAAGACCA	979	2233	GCUCUAGAGCAGGAAGACCA	979	2255	UGGUCCUCCUGCUJUGAGC	1303
2251	AAAGAAAAAGACAUUGGG	980	2251	AAAGAAAAAGACAUUGGG	980	2273	CCACGGCAAGUGCUUUUCU	1304
2269	GUCAAGGCAGCUCACAGUCC	981	2269	GUCAAGGCAGCUCACAGUCC	981	2291	GGACUGUGAGCUGCCUGAC	1305
2287	CUAGAGGCUGUGGGACCCA	982	2287	CUAGAGGCUGUGGGACCCA	982	2309	UGGGUGGCACACGGCUUAG	1306
2305	ACGAUCACAGGAACCUUG	983	2305	ACGAUCACAGGAACCUUG	983	2327	CCAGGUUJUCUGUGAUUGU	1307
2323	GAGAAUCAAGACGACAAGUA	984	2323	GAGAAUCAAGACGACAAGUA	984	2345	UACUJUGUGUCUGAUUCUC	1308
2341	AUJGGGGAAAGCAUCGAAAG	985	2341	AUJGGGGAAAGCAUCGAAAG	985	2363	CUUCGAUGCUUUCCCCAAU	1309
2359	GUICUCAUGACGGCAUCJUG	986	2359	GUICUCAUGACGGCAUCJUG	986	2381	CAGAUGCGCUGCAUGAGAC	1310
2377	GGGAAUCCCCCUCCACAGA	987	2377	GGGAAUCCCCCUCCACAGA	987	2399	UCUGGGAGGGGGAUUCCC	1311
2395	AUCAUGUGGUUAAAAGAU	988	2395	AUCAUGUGGUUAAAAGAU	988	2417	UAUCUUAAAACCACAUAGU	1312
2413	AAUGAGACCCUUJGUAGAAG	989	2413	AAUGAGACCCUUJGUAGAAG	989	2435	CUUCUACAAAGGGUCUCAUU	1313
2431	GACUCAGGCAUJGUAGA	990	2431	GACUCAGGCAUJGUAGA	990	2453	UCAAUACAAUGCCUGAGUC	1314

2449	AGGAUGGGAACCGGAACC	991	2449	AAGGAUGGGAACCGGAACC	991	2471	GGUUCGGGUUCCCAUCUU	1315
2467	CUCACUAUCCGAGAGUGA	992	2467	CUCACUAUCCGAGAGUGA	992	2489	UCACUCUGGGAUAGUGAG	1316
2485	AGGAAGGGAGACGAAGGCC	993	2485	AGGAAGGGAGACGAAGGCC	993	2507	GGCCUUCUGGUCCUCUCCU	1317
2503	CUCUACACUGCCAGGCAU	994	2503	CUCUACACUGCCAGGCAU	994	2525	AUGCCUGGGAGGUAGAG	1318
2521	UGCAGUGUUCUJGGCUGUG	995	2521	UGCAGUGUUCUJGGCUGUG	995	2543	CACAGCCAAGAACACUGCA	1319
2539	GCAAAAGUGGGAGGCAUUU	996	2539	GCAAAAGUGGGAGGCAUUU	996	2561	AAAUGCCUCCACUJUGC	1320
2557	UUCAUAAAUGGAAGGGGCC	997	2557	UUCAUAAAUGGAAGGGGCC	997	2579	GGGCACCUUCUAAUJUGAA	1321
2575	CAGGAAAAGACGAACUJGG	998	2575	CAGGAAAAGACGAACUJGG	998	2597	CCAAGUUCGUUCUJUCCUG	1322
2593	GAAAUCAUAAAUCUAGUAG	999	2593	GAAAUCAUAAAUCUAGUAG	999	2615	CUACUAGAAAUAUGAUUUUC	1323
2611	GGCACGGGGUGAUJGCCA	1000	2611	GGCACGGGGUGAUJGCCA	1000	2633	UGGCAAUACCGCCUGGCC	1324
2629	AUGUUCUUCUGGUACUUC	1001	2629	AUGUUCUUCUGGUACUUC	1001	2651	GAAGUAGCCAGAAAGAACAU	1325
2647	CUUGGUCAUCAUCCUACGGA	1002	2647	CUUGGUCAUCAUCCUACGGA	1002	2669	UCCGUAGGAUGAUGACAAG	1326
2665	ACCGGUAAAGGGCCAAUG	1003	2665	ACCGGUAAAGGGCCAAUG	1003	2687	CAUUGGCCCGCUUAAACGGU	1327
2683	GGAGGGGAACUGAACAG	1004	2683	GGAGGGGAACUGAACAG	1004	2705	CUGUCUUCAGUUCUCCUCC	1328
2701	GGCUACUUJGUCCAUCGUCA	1005	2701	GGCUACUUJGUCCAUCGUCA	1005	2723	UGACGAUGGACAAGUAGCC	1329
2719	AUGGAUCCAGAAGAACUCC	1006	2719	AUGGAUCCAGAAGAACUCC	1006	2741	GGAGUIUCAUCUGGAUCCAU	1330
2737	CCAUUJGGAAGAACAUJUG	1007	2737	CCAUUJGGAAGAACAUJUG	1007	2759	CACAAUGGUUCAUCAAUGG	1331
2755	GAACGACUGGCCUUAUGAUG	1008	2755	GAACGACUGGCCUUAUGAUG	1008	2777	CAUCAUAGGGAGGUUCGUUC	1332
2773	GCCAGCAAAUGGGAAUUCC	1009	2773	GCCAGCAAAUGGGAAUUCC	1009	2795	GGAAUUCCCAUUUGGUCC	1333
2791	CCAGAGAACGGCGUGAAGC	1010	2791	CCAGAGAACGGCGUGAAGC	1010	2813	GCUUUCAGCCGGGUUCUGGG	1334
2809	CUAGGUAAAGCCUJUGGCC	1011	2809	CUAGGUAAAGCCUJUGGCC	1011	2831	GGCCAAGAGGGCUUACCUAG	1335
2827	CGUGGUGCCUJUGGCCAAG	1012	2827	CGUGGUGCCUJUGGCCAAG	1012	2849	CUUGGCCAAAGGCCACACG	1336
2845	GUGAUJUGAAGCAGAUGCCU	1013	2845	GUGAUJUGAAGCAGAUGCCU	1013	2867	AGGCAUCUGGUUCAUUCAC	1337
2863	UUUGGAAUUGACAAGACAG	1014	2863	UUUGGAAUUGACAAGACAG	1014	2885	CUGUCUJGUUCAUUCAAA	1338
2881	GCAACUJGAGGAGACUAG	1015	2881	GCAACUJGAGGAGACUAG	1015	2903	CUACUGUCCUGCAAGUUGC	1339
2899	GCAGGUCAAAUGGUAGAAG	1016	2899	GCAGGUCAAAUGGUAGAAG	1016	2921	CUUCAACAUUUUGACUGC	1340
2917	GAAGGAGGAACACACAGUG	1017	2917	GAAGGAGGAACACACAGUG	1017	2939	CACUGUGGUUGGUCCUUC	1341
2935	GAAGCAUCGAGCUCUCAUG	1018	2935	GAAGCAUCGAGCUCUCAUG	1018	2957	ACAUAGAGGCUUAGGCUC	1342
2953	UCUGAACCUAAGAACCUCA	1019	2953	UCUGAACCUAAGAACCUCA	1019	2975	UGAGGAUCUJUGGUUCAGA	1343
2971	AUUCAUAUJGGGUACCAUC	1020	2971	AUUCAUAUJGGGUACCAUC	1020	2993	GAUGGGUACCCAAUUGAAU	1344
2989	CUCAAUGGGGUACCCUUC	1021	2989	CUCAAUGGGGUACCCUUC	1021	3011	GAAGGGUAGCCACAUJUGAG	1345
3007	CUAGGUGCCUGGUACCAAGC	1022	3007	CUAGGUGCCUGGUACCAAGC	1022	3029	GCUGGGGUACAGGGACCUAG	1346
3025	CCAGGAGGGCCACUCAUGG	1023	3025	CCAGGAGGGCCACUCAUGG	1023	3047	CCAUGGAGGGCCUCUCCUGG	1347
3043	GUGAUJUGGAAUUCUGCA	1024	3043	GUGAUJUGGAAUUCUGCA	1024	3065	UGCAGAAUUCACAAUCAC	1348
3061	AAAUUUGGAAACCUGCCA	1025	3061	AAAUUUGGAAACCUGCCA	1025	3083	UGGACAGGUUUCACAAUUU	1349
3079	ACUUACCUGAGGGAGCAAGA	1026	3079	ACUUACCUGAGGGAGCAAGA	1026	3101	UCUJGCUCCUCAGGUAGU	1350

3097	AGAAUAGAAUUJUGUCCCU	1027	3097	AGAAUAGAAUUJUGUCCCU	1027	3119	AGGGGACAAAUCUUUCU	1351
3115	UACAAGACCAAAGGGCAC	1028	3115	UACAAAGACCAAAGGGCAC	1028	3137	GUGCCCUUUGGUUCUUGUA	1352
3133	CGAUUCCGUCAAGGAAAG	1029	3133	CGAUUCCGUCAAGGAAAG	1029	3155	CUUCCCUUUGACGGAAUCG	1353
3151	GACUACGUUGGAGCAUCC	1030	3151	GACUACGUUGGAGCAUCC	1030	3173	GGAUUUCUCCAACGUAGUC	1354
3169	CGUGGGAUCUGAACGGC	1031	3169	CGUGGGAUCUGAACGGC	1031	3191	GCGUUUCUCAUCACAGG	1355
3187	CGCUUUGGACAGCAUCACCA	1032	3187	CGCUUUGGACAGCAUCACCA	1032	3209	UGGUGAUGCUGUCCAAGCG	1356
3205	AGUAGCCAGAGCUAGCCA	1033	3205	AGUAGCCAGAGCUAGCCA	1033	3227	UGGCUGAGGCUCUGGCCUACU	1357
3223	AGCUCUGGAAUJUGGGAGG	1034	3223	AGCUCUGGAAUJUGGGAGG	1034	3245	CCUCCACAAAUCAGAGCU	1358
3241	GAGAAGUCUCCUCAGUGAUG	1035	3241	GAGAAGUCUCCUCAGUGAUG	1035	3263	CAUCACUGAGGGACUUCUC	1359
3259	GUAGAAGAAGAGGAAGCUC	1036	3259	GUAGAAGAAGAGGAAGCUC	1036	3281	GAGCUUUCUUCUUCUAC	1360
3277	CCUGAAGAUCUGUAUAGG	1037	3277	CCUGAAGAUCUGUAUAGG	1037	3299	CCUUUAACAGAACUUCUAGG	1361
3295	GAUCUCCUGACCUUGGAGC	1038	3295	GAUCUCCUGACCUUGGAGC	1038	3317	GCUCCAAGGUCAGGAAGUC	1362
3313	CAUCUCAUCUGUUACAGCU	1039	3313	CAUCUCAUCUGUUACAGCU	1039	3335	AGCUGUAACAGAUGAGAUG	1363
3331	UCCCAAGUGGCUAAGGGCA	1040	3331	UCCCAAGUGGCUAAGGGCA	1040	3353	UGCCCUUAGGCCACUJUGGAA	1364
3349	AUGGAGGUUCUJUGCAUCGC	1041	3349	AUGGAGGUUCUJUGCAUCGC	1041	3371	GCGAUGCCAAGAACUCCAU	1365
3367	CGAAAGGUUAUCCACAGGG	1042	3367	CGAAAGGUUAUCCACAGGG	1042	3389	CCCUUGGUUAACACUUUCG	1366
3385	GAACCUGGGGCACGAAUA	1043	3385	GAACCUGGGGCACGAAUA	1043	3407	UAUUUCUGGCCACGGUC	1367
3403	AUCCUCUUAUCGGAGAAGA	1044	3403	AUCCUCUUAUCGGAGAAGA	1044	3425	UCUUCUCCGUAUAGGGAU	1368
3421	AACGUUGGUAAAUCUGUG	1045	3421	AACGUUGGUAAAUCUGUG	1045	3443	CACAGAUUUUACACGUU	1369
3439	GAUCUUGGUUGGCCGGG	1046	3439	GAUCUUGGUUGGCCGGG	1046	3461	CCCGGGCCAAGCCAAAGUC	1370
3457	GAUUUUUAAAAGAUCCAG	1047	3457	GAUUUUUAAAAGAUCCAG	1047	3479	CUGGAUCUUUAAAUAUUC	1371
3475	GAUUAUGUCAGAAAAGGAG	1048	3475	GAUUAUGUCAGAAAAGGAG	1048	3497	CUCCUUUCUGACAUAAUC	1372
3493	GAUGCUUGGCCCUUUGA	1049	3493	GAUGCUUGGCCCUUUGA	1049	3515	UCAAAGGGAGGGGAGCAUC	1373
3511	AAAUGGAUGGCCAGAAA	1050	3511	AAAUGGAUGGCCAGAAA	1050	3533	UUCUCGGGGCCAUCUCAUUU	1374
3529	ACAAUUUUJUGACAGUGU	1051	3529	ACAAUUUUJUGACAGUGU	1051	3551	ACACUCUGUAAAUAUUGU	1375
3547	UACACAAUCCAGUGACG	1052	3547	UACACAAUCCAGUGACG	1052	3569	CGUCACUCUGGGAUUGUGUA	1376
3565	GUCLUGGUUCUJUGGUUU	1053	3565	GUCLUGGUUCUJUGGUUU	1053	3587	AAACACAAAAAGACAGAGC	1377
3583	UJGGCUGGGGAAAUUUUU	1054	3583	UJGGCUGGGGAAAUUUUU	1054	3605	AAAAUUUUUCCACAGCAA	1378
3601	UCCUUAGGUUGGUUCUCCAU	1055	3601	UCCUUAGGUUGGUUCUCCAU	1055	3623	AUGGAGAAGGCACCUAAGGA	1379
3619	UAUCCUGGGGUAAAAGAUU	1056	3619	UAUCCUGGGGUAAAAGAUU	1056	3641	CAAUCUUUACCCCCAGGAAU	1380
3637	GAUGAAGAAUUUUJUGAGGC	1057	3637	GAUGAAGAAUUUUJUGAGGC	1057	3659	GCCUACAAAAUUUCUCAUC	1381
3655	CGAUUJUGAAAGGAACUA	1058	3655	CGAUUJUGAAAGGAACUA	1058	3677	UAGUUCUUCUUCUAAUCG	1382
3673	AGAAUAGAGGGCCCUJGAU	1059	3673	AGAAUAGAGGGCCCUJGAU	1059	3695	AAUCAGGGGCCCUAUUCU	1383
3691	UAUACUACACCAGAAAUGU	1060	3691	UAUACUACACCAGAAAUGU	1060	3713	ACAUUUUCUGGUUGUAGUAUA	1384
3709	UACCAAGACCAUGCUGGACU	1061	3709	UACCAAGACCAUGCUGGACU	1061	3731	AGUCCAGACCAUGGUUGGUUA	1385
3727	UGCUGGCACGGGGAGCCCCA	1062	3727	UGCUGGCACGGGGAGCCCCA	1062	3749	UGGGCUCCCCCGUGCCAGCA	1386

3745	AGUCAGAGACCCACGUUUU	1063	3745	AGUCAGAGACCCACGUUUU	1063	3767	AAAACGUJGGGUCCUCUGACU	1387
3763	UCAGAGUJGGGUACAUU	1064	3763	UCAGAGUJGGGUACAUU	1064	3785	AAUGUJUCCACCAACUCUGA	1388
3781	UJGGGAAAUCUCUJGCAAG	1065	3781	UJGGGAAAUCUCUJGCAAG	1065	3803	CUUCCAGAGAGAUUUCCAA	1389
3799	GCUAUAGCUAGCGAUG	1066	3799	GCUAUAGCUAGCGAUG	1066	3821	CAUCCUGCUGAGCAUUAGC	1390
3817	GGCAAAGACUACAUJGUUC	1067	3817	GGCAAAGACUACAUJGUUC	1067	3839	GAACAUGUAGCUUJGGCC	1391
3835	CUUCCGUAUACAGACUU	1068	3835	CUUCCGUAUACAGACUU	1068	3857	AAGUCUCUGUAUUCGGAAAG	1392
3853	UUGAGCAUGGAAGAGGAU	1069	3853	UUGAGCAUGGAAGAGGAU	1069	3875	AAUCCUCUCCAUUGCUCAA	1393
3871	UCUGGACUCUCUCUGCCUA	1070	3871	UCUGGACUCUCUCUGCCUA	1070	3893	UAGGCAGAGAGGUCCAGA	1394
3889	ACCUCACCUJGUUUCUGUA	1071	3889	ACCUCACCUJGUUUCUGUA	1071	3911	UACAGGAACAGGGUAGGGU	1395
3907	AUGGAGGGAGGAAGGUAU	1072	3907	AUGGAGGGAGGAAGGUAU	1072	3929	AUACUUUCCUCUCCUCCAU	1396
3925	UGUGACCCAAUUCAUU	1073	3925	UGUGACCCAAUUCAUU	1073	3947	AUAGGAUUJGGGUUCACAA	1397
3943	UAUGACAAACACAGCAGAA	1074	3943	UAUGACAAACACAGCAGAA	1074	3965	UUCUCUGCUGGUUGUCAUA	1398
3961	AUCAGUCAGUAUCUGCAGA	1075	3961	AUCAGUCAGUAUCUGCAGA	1075	3983	UCUGCAGAUACUGACUGAU	1399
3979	AACAGUAAAGCAGAGGCC	1076	3979	AACAGUAAAGCAGAGGCC	1076	4001	GGCUCUJUUCGCUUACUGUU	1400
3997	CGGCCUGUGAGGUAAAAA	1077	3997	CGGCCUGUGAGGUAAAAA	1077	4019	UUUUUACACUCACAGGCCG	1401
4015	ACAUUUGAAGUAUCCCGU	1078	4015	ACAUUUGAAGUAUCCCGU	1078	4037	ACGGGAUJAUCUAAAUGU	1402
4033	UAGAAAGAACCGAGAUAA	1079	4033	UAGAAAGAACCGAGAUAA	1079	4055	UUACUUUCUGGUUUCUCAA	1403
4051	AAAGUAAUCCAGAUGACA	1080	4051	AAAGUAAUCCAGAUGACA	1080	4073	UGUCAUCUGGGAUJACUUU	1404
4069	AACCAGAGGGACAGUGGU	1081	4069	AACCAGAGGGACAGUGGU	1081	4091	UACCACUGUCCGUCUGGUU	1405
4087	AUGGUJUCUJGCUCAAG	1082	4087	AUGGUJUCUJGCUCAAG	1082	4109	CUUCUGAGGCAAGAACCAU	1406
4105	GAUCUGAAAACUJUGGAAG	1083	4105	GAUCUGAAAACUJUGGAAG	1083	4127	CUUCCAAAGUUUUUAGCUC	1407
4123	GACAGAAACAAUUAUCUC	1084	4123	GACAGAAACAAUUAUCUC	1084	4145	GAGAUAAUJGGGUUCUGUC	1408
4141	CCAUCUJUJGGUGGAUGG	1085	4141	CCAUCUJUJGGUGGAUGG	1085	4163	CCAUUCCACCAAAAGAUGG	1409
4159	GUGCCAGAACAGAGGG	1086	4159	GUGCCAGAACAGAGGG	1086	4181	CCUCGCUUJUGCUGGGCAC	1410
4177	GAUCUGUGGGCAUCUGAAG	1087	4177	GAUCUGUGGGCAUCUGAAG	1087	4199	CUUCAGAUGGCCACAGACUC	1411
4195	GGCUAAACCCAGACAAGCG	1088	4195	GGCUAAACCCAGACAAGCG	1088	4217	CCUUCUGCUGGUUJUGAGCC	1412
4213	GGCUACCAUCGGGUAAUC	1089	4213	GGCUACCAUCGGGUAAUC	1089	4235	GAUAUCCGGACUGGUAGGCC	1413
4231	CAUCUCGAUGACACAGACA	1090	4231	CAUCUCGAUGACACAGACA	1090	4253	UGUCUGUGUCAUCGGAGUG	1414
4249	ACCCACGGGUACUCCAGUG	1091	4249	ACCCACGGGUACUCCAGUG	1091	4271	CACUGGGAGUACACGGUGGU	1415
4267	GAAGGAAGCAGAAUUUAAA	1092	4267	GAAGGAAGCAGAAUUUAAA	1092	4289	UJAAAAGUUCUGCUUCCUC	1416
4285	AAGCUCGAUGAGAUJGGAG	1093	4285	AAGCUCGAUGAGAUJGGAG	1093	4307	CUCCAAUCUCUCAUCAGCUU	1417
4303	GUJGCAAACCCGGUAGCACAG	1094	4303	GUJGCAAACCCGGUAGCACAG	1094	4325	CUGUGCUACCGGUUJUGGCAC	1418
4321	GCCCCAGAUUCUCCAGCCUG	1095	4321	GCCCCAGAUUCUCCAGCCUG	1095	4343	CAGGCUGGGAGAAUCUGGGC	1419
4339	GACUCGGGGACACACUGA	1096	4339	GACUCGGGGACACACUGA	1096	4361	UCAGUGGGGUCCCGAGUC	1420
4357	AGCUCUCUCCUCGUUUAAA	1097	4357	AGCUCUCUCCUCGUUUAAA	1097	4379	UJUAAAACAGGGAGGAGGU	1421
4375	AAGGAAGCAUCCACACCCC	1098	4375	AAGGAAGCAUCCACACCCC	1098	4397	GGGGUGGUGGAUGGUUCUU	1422

4393	CAACUCCCGGACAUCACAU	1099	4393	CAACUCCCGGACAUCACAU	1099	4415	AUGUGAUGUCGGGAGUUG	1423
4411	UGAGAGGGUCUGCUAGAUU	1100	4411	UGAGAGGGUCUGCUAGAUU	1100	4433	AAUCUGAGCGACCUUCUA	1424
4429	UUUGGAAGGUUGGUUCUUC	1101	4429	UUUGGAAGGUUGGUUCUUC	1101	4451	GAAAAGAACACIUCAAA	1425
4447	CCACCCAGGAAAGUAGCC	1102	4447	CCACCCAGGAAAGUAGCC	1102	4469	GGCUACUUCUCCUGCUGGG	1426
4465	CGCAUUUUGAUUUCAUUC	1103	4465	CGCAUUUUGAUUUCAUUC	1103	4487	GAAAUGAAAAUCAAAUUGCG	1427
4483	CGACAACAGAAAAAGGACC	1104	4483	CGACAACAGAAAAAGGACC	1104	4505	GGUCCUUUUUCGUUGUUCG	1428
4501	CUCGGACUGCCAGGGGCCA	1105	4501	CUCGGACUGCCAGGGGCCA	1105	4523	UGGCUCUCCUGCAGUCCGAG	1429
4519	AGUCUUUCUAGGCAUAUCU	1106	4519	AGUCUUUCUAGGCAUAUCU	1106	4541	AGGAUUAUGCCUUAAGACU	1430
4537	UGGAAGAGGGCUUUGUGACCC	1107	4537	UGGAAGAGGGCUUUGUGACCC	1107	4559	GGGUACACAGGCCUUCUCCA	1431
4555	CAAGAAUGUGUCUGUGUCU	1108	4555	CAAGAAUGUGUCUGUGUCU	1108	4577	AGACACAGACACAUUCUUG	1432
4573	UUCUCCCAUGGUUGACCUG	1109	4573	UUCUCCCAUGGUUGACCUG	1109	4595	CAGGUCAACACUGGGAGAA	1433
4591	GAUCCUCUUUUUUCAUCA	1110	4591	GAUCCUCUUUUUUCAUCA	1110	4613	UGAAUAGAAAAAGGGAUC	1434
4609	AUUAAAAGCAUUAUCAU	1111	4609	AUUAAAAGCAUUAUCAU	1111	4631	AUGAAUAAUGCUUUUUAAAU	1435
4627	UGCCCCUCCUGGGGGUCUC	1112	4627	UGCCCCUCCUGGGGGUCUC	1112	4649	GAGACCCGAGCAGGGGA	1436
4645	CACCAUGGGUUUUAGAACAA	1113	4645	CACCAUGGGUUUUAGAACAA	1113	4667	UUGUUCAAAACCCAUUGGUG	1437
4663	AAGAGCCUUCAAGCAUAGGC	1114	4663	AAGAGCCUUCAAGCAUAGGC	1114	4685	GCCAUUGCUUAGCUCUU	1438
4681	CCCCAUCCUCAAAAGAAGUA	1115	4681	CCCCAUCCUCAAAAGAAGUA	1115	4703	UACUUCUUCUUGAGGAUGGG	1439
4699	AGCAGUACCCUGGGAGCUG	1116	4699	AGCAGUACCCUGGGAGCUG	1116	4721	CAGCCUCCCCAGGUACUGCU	1440
4717	GACACUUUCGUAAAACUAG	1117	4717	GACACUUUCGUAAAACUAG	1117	4739	CUAGUUUUACAGAAGUGUC	1441
4735	GAAGAUAAAACCAGGCAACG	1118	4735	GAAGAUAAAACCAGGCAACG	1118	4757	CGUUGCCUGGGUIUAUCUUC	1442
4753	GUAAAGUUGUUCGAGGUUG	1119	4753	GUAAAGUUGUUCGAGGUUG	1119	4775	CAACACCUUCGAAACCUUAC	1443
4771	GAAGAUGGGAAAGGAAUUGC	1120	4771	GAAGAUGGGAAAGGAAUUGC	1120	4793	GCAAUAUCCUUCCAUUCUUC	1444
4789	CAGGGCUGAGCUAUCCAA	1121	4789	CAGGGCUGAGCUAUCCAA	1121	4811	UJGGAUAGACUCAGCCUG	1445
4807	AGAGGCCUUUJGUUAGGACG	1122	4807	AGAGGCCUUUJGUUAGGACG	1122	4829	CGUCCUAAAAGCCUCU	1446
4825	GUUGGUCCCCAAGGCCAAGCC	1123	4825	GUUGGUCCCCAAGGCCAAGCC	1123	4847	GGCUUJGGCUUJGGGACCCAC	1447
4843	CUUAAGUGGGAAUUCGGA	1124	4843	CUUAAGUGGGAAUUCGGA	1124	4865	UCCGAUAIUCCACCUUAAG	1448
4861	AUUGAUAGAAAGGAAGACU	1125	4861	AUUGAUAGAAAGGAAGACU	1125	4883	AGUCUUCUUUCUUAUCUAU	1449
4879	UAAAGGUUACCUUUCGUJGG	1126	4879	UAAAGGUUACCUUUCGUJGG	1126	4901	CCAAAGGCAAGGUAAAGUUA	1450
4897	GAGAGUACUGGGAGCCUGCA	1127	4897	GAGAGUACUGGGAGCCUGCA	1127	4919	UGCAGGGCUCCAGUACUCUC	1451
4915	AAAUGCAUUGGUUJUGCU	1128	4915	AAAUGCAUUGGUUJUGCU	1128	4937	GAGCAAAACACAAUGCAUUU	1452
4933	CUGGGUGGGGGCAUGG	1129	4933	CUGGGUGGGGGCAUGG	1129	4955	CCAUGCCCCACCUCCACCG	1453
4951	GGGUCUGUUCUAGAAUGUA	1130	4951	GGGUCUGUUCUAGAAUGUA	1130	4973	UACAUUUUCAGAAGACGCC	1454
4969	AAAGGGUUUCAGACGGGGUU	1131	4969	AAAGGGUUUCAGACGGGGUU	1131	4991	AACCCCGUGUGAACCCUUU	1455
4987	UUCUGGUUUUAGAAGGUUG	1132	4987	UUCUGGUUUUAGAAGGUUG	1132	5009	CAACCUUCUAAAACAGAA	1456
5005	GCGGUGUUCUUCGAGUUGG	1133	5005	GCGGUGUUCUUCGAGUUGG	1133	5027	CCCAACUCGAAAGAACACGCC	1457
5023	GUAAAAGGUAGAGUUCGUUG	1134	5023	GUAAAAGGUAGAGUUCGUUG	1134	5045	CAACGAACUCUACUUJAGC	1458

5041	GUGCUGUUUCGACUCCUA	1135	5041	GUGGUGUUUCGACUCCUA	1135	5063	UAGGAGUCAGAAAACAGCAC	1459
5059	AUGAGAGUUCCUCCAGA	1136	5059	AAUGAGAGUUCCUCCAGA	1136	5081	UCUGGAAGGAACUCUCAUU	1460
5077	ACCGUUAGCUCGUCCUUG	1137	5077	ACCGUUAGCUCGUCCUUG	1137	5099	CAAGGAGACGCCAACGGU	1461
5095	GCCAAGCCCCAGGAAGAAA	1138	5095	GCCAAGCCCCAGGAAGAAA	1138	5117	UUUCUCCUGGGCUUGGC	1462
5113	AUGAUGCAGCUCUGGCUC	1139	5113	AAUGAUGCAGCUCUGGCUC	1139	5135	GAGCCAGAGCUGCAUCAUU	1463
5131	CCUUGUCUCCAGGUGAU	1140	5131	CCUUGUCUCCAGGUGAU	1140	5153	AUCAGCCUGGGAGACAAGG	1464
5149	UCCUUUAUUCAGAAUACCA	1141	5149	UCCUUUAUUCAGAAUACCA	1141	5171	UGGUAAUUCUGAAUAAAAGGA	1465
5167	ACAAAAGAAAGGACAUUCAG	1142	5167	ACAAAAGAAAGGACAUUCAG	1142	5189	CUGAAUGGUCCUUUCUUUGU	1466
5185	GCUCAAGGCUCCCUGCCGU	1143	5185	GCUCAAGGCUCCCUGCCGU	1143	5207	ACGGCAGGGAGCCUUGGAGC	1467
5203	UGUUGAAGAGUUUCGACUG	1144	5203	UGUUGAAGAGUUUCGACUG	1144	5225	CAGUCAGAACUCUUCUACA	1468
5221	GCACAAACACGCCUUCUGGU	1145	5221	GCACAAACACGCCUUCUGGU	1145	5243	ACCGAGCUGGUUCUUGUGC	1469
5239	UUUCUUCUGGAAUGAAUAC	1146	5239	UUUCUUCUGGAAUGAAUAC	1146	5261	GUAAUCAUUCAGAAAGAAA	1470
5257	CCCUCAUACUGGUCCUGAU	1147	5257	CCCUCAUACUGGUCCUGAU	1147	5279	AUCAGGACAGAUAGUGGG	1471
5275	UGUGAUAUUGUCUGAGACUG	1148	5275	UGUGAUAUUGUCUGAGACUG	1148	5297	CAGUCUCAGACAUUAUCACA	1472
5293	GAAUGGGGAGGUUCAUAG	1149	5293	GAAUGGGGAGGUUCAUAG	1149	5315	CAUUGAACCUCCCGCAUUC	1473
5311	GUGAACGCUUGUGUGUGU	1150	5311	GUGAACGCUUGUGUGUGU	1150	5333	ACACCCACACAGCUUCAC	1474
5329	UCAAAGUUUCAGGAAGGAU	1151	5329	UCAAAGUUUCAGGAAGGAU	1151	5351	AUCCUUCCUGAAACUUUGA	1475
5347	UUUUACCCUUUGUUUCUUC	1152	5347	UUUUACCCUUUGUUUCUUC	1152	5369	GAAGAACAAAAGGUAAA	1476
5365	CCCCCUGUCCCCAACCCAC	1153	5365	CCCCCUGUCCCCAACCCAC	1153	5387	GUGGGUUGGGGACAGGGGG	1477
5383	CUCUCACCCCGCAACCCAU	1154	5383	CUCUCACCCCGCAACCCAU	1154	5405	AUGGGUUGGGGGUGAGAG	1478
5401	UCAGUAUUUAGUUUJUG	1155	5401	UCAGUAUUUAGUUUJUG	1155	5423	CAAAUACUAAAUAUCUGA	1479
5419	GGCCUCUACUCCAGUAAAC	1156	5419	GGCCUCUACUCCAGUAAAC	1156	5441	GUUUACUGGAGUAGGGCC	1480
5437	CCUGAUUUGGUUJUGUUCAC	1157	5437	CCUGAUUUGGUUJUGUUCAC	1157	5459	GUGAACAAAACCCAAUCAGG	1481
5455	CUCUCUGAAUGAUUUJUAG	1158	5455	CUCUCUGAAUGAUUUJUAG	1158	5477	CUAAUAAUCAUUCAGAGAG	1482
5473	GCCAGACUCAAAAUUU	1159	5473	GCCAGACUCAAAAUUU	1159	5495	AUAAAUUUGAAGUCUGGC	1483
5491	UUUAUAGCCAAAUUAAA	1160	5491	UUUAUAGCCAAAUUAAA	1160	5513	UUUAUAAUUGGGCUUAUAAA	1484
5509	ACAUCUAUJGUUAUJUUA	1161	5509	ACAUCUAUJGUUAUJUUA	1161	5531	UUAAUAAUACAAUJGAUGU	1485
5527	AGACUUUUACAUUAGAG	1162	5527	AGACUUUUACAUUAGAG	1162	5549	CUCUUAUUGUUAAAAGUCU	1486
5545	GCUAAUUCUACUGUUUU	1163	5545	GCUAAUUCUACUGUUUU	1163	5567	AAAAAUUCAGUAGAAAAGC	1487
5563	UGCCCUUUGUCUGCUUU	1164	5563	UGCCCUUUGUCUGCUUU	1164	5585	AAAGGGACAGAACGGGCA	1488
5581	UUUUUCAAAAAGAAAUG	1165	5581	UUUUUCAAAAAGAAAUG	1165	5603	CAUUUUUUUUUUUAGAAAAA	1489
5599	GUUUUUUUUGUUUGUACC	1166	5599	GUUUUUUUUGUUUGUACC	1166	5621	GGUACCAAAACAAAAACAC	1490
5617	CAUAGUGUGAAAUGUGGG	1167	5617	CAUAGUGUGAAAUGUGGG	1167	5639	CCCAGCAUUCACCUAUG	1491
5635	GAACAAUGACUUAAGACA	1168	5635	GAACAAUGACUUAAGACA	1168	5657	UGUCUUAAUAGCUAUGUUC	1492
5653	AUGCUAUGGCCACAUUAU	1169	5653	AUGCUAUGGCCACAUUAU	1169	5675	AAUAAUAGUGGCCACAUUAU	1493
5671	UUUAUAGUCUGUUUAUGUAG	1170	5671	UUUAUAGUCUGUUUAUGUAG	1170	5693	CUACAUAAACAGACUUAUA	1494

5689	GAAACAAAGUAAUAAUAAU	1171	5689	GAAACAAAGUAAUAAUAAU	1171	5711	AUAUAUUAUACAUUUGUUUC	1495
5707	UAAAAGCCUUAUAAUAAU	1172	5707	UAAAAGCCUUAUAAUAAU	1172	5729	CAUUAUUAUAAAGGCCUUUA	1496
5725	GAACUUUUGUACUUAUCA	1173	5725	GAACUUUUGUACUUAUCA	1173	5747	UGUGAAUAGUACAAAGUUC	1497
5743	AUUIJUGUAUCAGUAUUAU	1174	5743	AUUIJUGUAUCAGUAUUAU	1174	5765	CAUAAUACUGAUACAAAAAU	1498
5761	GUAGCAUAACAAAGGUCAU	1175	5761	GUAGCAUAACAAAGGUCAU	1175	5783	AUGACCUUJGUUAUJGUAC	1499
5779	UAAUUGCUUUCAGCAAUUGA	1176	5779	UAAUUGCUUUCAGCAAUUGA	1176	5801	UCAAUUGCUGAAAGCAUUA	1500
5797	AUGUCAUUUUUAUAAAGAA	1177	5797	AUGUCAUUUUUAUAAAGAA	1177	5819	UUCUUAUAAAAGACAU	1501
5812	AGAACAUUGAAAAACUUGA	1178	5812	AGAACAUUGAAAAACUUGA	1178	5834	UCAAGUUUUUCAUUGUUCU	1502

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Pos	Target Sequence	Seq ID	UpPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1	ACCCACGGCCAGCGCCGG	1503	1	ACCCACGGCCAGCGCCGG	1503	23	CGGGCCGCCUGGCCUGGGU	1750
19	GAGAUGCAGGGGGGCCG	1504	19	GAGAUGCAGGGGGGCCG	1504	41	CGGGCCGCCUGGCCUGCAUCUC	1751
37	GCGCUGUGCCUGGCACUGU	1505	37	GCGCUGUGCCUGGCACUGU	1505	59	ACAGUCCAGGCACAGCGC	1752
55	UGGCUCUGCCUGGGACUCC	1506	55	UGGCUCUGCCUGGGACUCC	1506	77	GGAGUCCAGGCAGAGCCA	1753
73	CGGGACGGCCUGGUGAGUG	1507	73	CGGGACGGCCUGGUGAGUG	1507	95	CAUCACCCAGGCCUGCCAG	1754
91	GACUACUCUCAUGACCCCC	1508	91	GACUACUCUCAUGACCCCC	1508	113	GGGGGGUCAUGGAGUAGUC	1755
109	CCGACCUUUGAACAUACGG	1509	109	CCGACCUUUGAACAUACGG	1509	131	CCGUGAUGUUAAGGUCCGG	1756
127	GAGGAGUCACAGGUCAUCC	1510	127	GAGGAGUCACAGGUCAUCC	1510	149	CGAUGACGGUGUGACUCCUC	1757
145	GACACCGGGUGACAGCUGU	1511	145	GACACCGGGUGACAGCUGU	1511	167	ACAGGGCUGUCACCGGUGUC	1758
163	UCCAUCUCUCCUGAGGGAC	1512	163	UCCAUCUCUCCUGAGGGAC	1512	185	GUCCCCUCCUGGAGAUGGA	1759
181	CAGCACCCCCCUCGAGUGGG	1513	181	CAGCACCCCCCUCGAGUGGG	1513	203	CCCCACUCUGGGGGUGGUGCUG	1760
199	GUUUGGCCAGGCUACAG	1514	199	GUUUGGCCAGGCUACAG	1514	221	CCUGAGCUCCUGCCCAAGC	1761
217	GGGGCCGCCAGCACGGAG	1515	217	GGGGCCGCCAGCACGGAG	1515	239	CUCGGGUGGGCUGGCCUC	1762
235	GACAAGGACAGCGAGGACA	1516	235	GACAAGGACAGCGAGGACA	1516	257	UGUCCUCUGCUGCUUUGUC	1763
253	ACGGGGGGUGGUGCGAGACU	1517	253	ACGGGGGGUGGUGCGAGACU	1517	275	AGUCUCGGCACCACCCGU	1764
271	UGCGAGGGCACAGACGCCA	1518	271	UGCGAGGGCACAGACGCCA	1518	293	UGGCGUUCUGGCCUCUGCA	1765
289	AGGCCCUUACUGCAAGGUU	1519	289	AGGCCCUUACUGCAAGGUU	1519	311	ACACCUUUCAGUAGGGCCU	1766
307	UUGGUGGUGGCCACGGGUAC	1520	307	UUGGUGGUGGCCACGGGUAC	1520	329	GUACCUUUGGUGGAGGCAA	1767
325	CAUGCCAAACGACACAGGCA	1521	325	CAUGCCAAACGACACAGGCA	1521	347	UGCCUGUGUGUGGGCAUG	1768
343	AGCUACGCUUCGUACUACA	1522	343	AGCUACGCUUCGUACUACA	1522	365	UGUAGUAGGAGGUAGGCCU	1769
361	AGUACAUCAAGGCACGCA	1523	361	AGUACAUCAAGGCACGCA	1523	383	UGCGUGGCCUUAGGUACUU	1770

379	AUCGAGGGCACCGGGCG	1524	379	AUCGAGGGCACCGGGCG	1524	401	CGGCCGUGGGGCCUCGGAU	1771
397	GCCAGCUCCUACGGUUCG	1525	397	GCCAGCUCCUACGGUUCG	1525	419	CGAACACGUAGGAGCUGGC	1772
415	GUGGAGGACUUUAGCAGC	1526	415	GUGGAGGACUUUAGCAGC	1526	437	GCUGCUCAAAGUCUCUCAC	1773
433	CCAUUCAUCAACAAGCCUG	1527	433	CCAUUCAUCAACAAGCCUG	1527	455	CAGGCUGUUGUAUGGAAUGG	1774
451	GACACGCUUUGGUCAACA	1528	451	GACACGCUUUGGUCAACA	1528	473	UGUUGACCAAGGGGUGUC	1775
469	AGGAAGGGAGCCAUUGGG	1529	469	AGGAAGGGAGCCAUUGGG	1529	491	CCACAUUGGUCCUUCUCCU	1776
487	GUGCCCUGUCUGGUUCCA	1530	487	GUGCCCUGUCUGGUUCCA	1530	509	UGGACACCAAGACAGGGCAC	1777
505	AUCCCCGCCUCAUGUCA	1531	505	AUCCCCGCCUCAUGUCA	1531	527	UGACAUUUGAGGGGGGAU	1778
523	ACGCUGGCCUCGCAAAAGCU	1532	523	ACGCUGGCCUCGCAAAAGCU	1532	545	AGCUUUGCGAGGCCAGCGU	1779
541	UCGGUGGUGGGCCAGACG	1533	541	UCGGUGGUGGGCCAGACG	1533	563	CGUCUGGCCACAGCACCGA	1780
559	GGGCAGGGGGGGGGGG	1534	559	GGGCAGGGGGGGGGGG	1534	581	CCACACCCACCUCCUGGCC	1781
577	GAUGACCCGGGGGGCAUGC	1535	577	GAUGACCCGGGGGGCAUGC	1535	599	GCAUGCCCCGGGGGUCAUC	1782
595	CUCGUGGUCCACGCCACUGC	1536	595	CUCGUGGUCCACGCCACUGC	1536	617	GCAGUGGGGUGGGACACGGAG	1783
613	CUGCACGGAUGGCCUGUACC	1537	613	CUGCACGGAUGGCCUGUACC	1537	635	GGUACAGGGCAUCCUGGAG	1784
631	CUGCAGUGGGAGACCACCU	1538	631	CUGCAGUGGGAGACCACCU	1538	653	AGGUGGUCCUGGCACUGCAG	1785
649	UGGGGAGGACGGACUUC	1539	649	UGGGGAGGACGGACUUC	1539	671	GGAAUGGUCCUGGUCCCCA	1786
667	CUUCCAACCCCUUCUGG	1540	667	CUUCCAACCCCUUCUGG	1540	689	CCAGGAAGGGGUUGGAAAG	1787
685	GUGCACAUCAACGGAAACG	1541	685	GUGCACAUCAACGGAAACG	1541	707	CGUUGCCUGUGAUGUGGCAC	1788
703	GAGCUCUAAUGACAUCCAGC	1542	703	GAGCUCUAAUGACAUCCAGC	1542	725	GCUGGAUGGUCAUAGGCCUC	1789
721	CUGUUGCCAGGAAGUCGC	1543	721	CUGUUGCCAGGAAGUCGC	1543	743	GCGACUUCUGGGCAACAG	1790
739	CUGGAGCUGUGGUAGGGG	1544	739	CUGGAGCUGUGGUAGGGG	1544	761	CCCCUACCCAGCAGCUCCAG	1791
757	GAGAAGCUGGUCCUCAACU	1545	757	GAGAAGCUGGUCCUCAACU	1545	779	AGUUGAGGACCACGUUCUC	1792
775	UGCACCGUGGGGUJGAGU	1546	775	UGCACCGUGGGGUJGAGU	1546	797	ACUCAGCCCACACGGUGCA	1793
793	UUUAACUCAAGGUACCCU	1547	793	UUUAACUCAAGGUACCCU	1547	815	AGGUGACACCUAGGUAAA	1794
811	UUUGACUGGGACUACCCAG	1548	811	UUUGACUGGGACUACCCAG	1548	833	CUGGGUAGGUCCCCAGUAAA	1795
829	GGGAAGGGAGGGGG	1549	829	GGGAAGGGAGGGGG	1549	851	CCCGCUUCUGGCCUCUCC	1796
847	GUAAGUGGGGGCCGAGC	1550	847	GUAAGUGGGGGCCGAGC	1550	869	GCUCGGGCACCCACUUACC	1797
865	CGACGCUCCCCAACAGACCC	1551	865	CGACGCUCCCCAACAGACCC	1551	887	GGGUCGUUGGGAGCUGUC	1798
883	CACACAGAACUCUCCAGCA	1552	883	CACACAGAACUCUCCAGCA	1552	905	UGCUGGAGAGGUUCUGUGUG	1799
901	AUCCUGACCAUCCACAAAG	1553	901	AUCCUGACCAUCCACAAAG	1553	923	CGUUGUGGAUGGGUGAGGGAU	1800
919	GUAGGCCAGCACGACUCCG	1554	919	GUAGGCCAGCACGACUCCG	1554	941	CCAGGUCCUGUGCUGGCUGAC	1801
937	GGCUCGUAGUGUGGCAAGG	1555	937	GGCUCGUAGUGUGGCAAGG	1555	959	CCUUGCACACAUACGGAGCC	1802
955	GCCAAACAACGGCAUCCAGC	1556	955	GCCAAACAACGGCAUCCAGC	1556	977	CGUGGAUGGCCGUUGUUGGC	1803

973	CGAUUUCCCCGAGGCACCG	1557	973	CGAUUUCCCCGAGGCACCG	1557	995	CGUGGCUCUCCCCGAAUUCG	1804
991	GAGGUCAUUGUGCAUGAAA	1558	991	GAGGUCAUUGUGCAUGAAA	1558	1013	UUUCAUGCACAAUGACCUC	1805
1009	AAUCCCUUCAUCAGGUCG	1559	1009	AAUCCCUUCAUCAGGUCG	1559	1031	CGACCGUGAUGGAAGGGAUU	1806
1027	GAGUGGUCAAAAGGACCA	1560	1027	GAGUGGUCAAAAGGACCA	1560	1049	UGGGUCUUUGGCCACUC	1807
1045	AUCCUGGGGCCACGGCG	1561	1045	AUCCUGGGGCCACGGCG	1561	1067	UGCCGUGGCCUCAGGAGU	1808
1063	GGAGACGAGCUGGUGAAGC	1562	1063	GGAGACGAGCUGGUGAAGC	1562	1085	GUUUCACCAGCUCGUCC	1809
1081	CUGCCCGUGAAGCUGGCAG	1563	1081	CUGCCCGUGAAGCUGGCAG	1563	1103	CUGCCAGCUUACGGGCAG	1810
1099	GCGUACCCCCGGCCGAGU	1564	1099	GCGUACCCCCGGCCGAGU	1564	1121	ACUCGGGGGGGUACGCG	1811
1117	UUCAGUGGUACAGGAUG	1565	1117	UUCAGUGGUACAGGAUG	1565	1139	CAUCUGUACCCACUGGAA	1812
1135	GGAAAGGCACUGUCCCCC	1566	1135	GGAAAGGCACUGUCCCC	1566	1157	GCCCCGACAGUGCCUUC	1813
1153	CGCCACAGUCCACAUGCC	1567	1153	CGCCACAGUCCACAUGCC	1567	1175	GGGCAUGUGGACUGUGGCG	1814
1171	CUGGUGGUCAAGGAGGUGA	1568	1171	CUGGUGGUCAAGGAGGUGA	1568	1193	UCACCUCCUUGAGCACCAG	1815
1189	ACAGAGGCCAGCACAGGA	1569	1189	ACAGAGGCCAGCACAGGA	1569	1211	UGCCUUGGUGGCCUCUGU	1816
1207	ACCUACACCCUCGCCCCUGU	1570	1207	ACCUACACCCUCGCCCCUGU	1570	1229	ACAGGGGGAGGGUGUAGGU	1817
1225	UGGAACACCCUCGCCCCUG	1571	1225	UGGAACACCCUCGCCCCUG	1571	1247	GGCCAGCAGGGAGUUCCA	1818
1243	CUGAGGGCGAACAUAGCC	1572	1243	CUGAGGGCGAACAUAGCC	1572	1265	GGCUGAUGUUGGCCUCAG	1819
1261	CUGGAGCUGGGUGGAAUG	1573	1261	CUGGAGCUGGGUGGAAUG	1573	1283	CAUUCACCACGCCUCCAG	1820
1279	GUGCCCCCCCAGAUACAUG	1574	1279	GUGCCCCCCCAGAUACAUG	1574	1301	CAUGUAUCUGGGGGCAC	1821
1297	GAGAAGGGGCCCUCCUCC	1575	1297	GAGAAGGGGCCCUCCUCC	1575	1319	GGGAGGGGCCUCUUCUC	1822
1315	CCCAGCAUCUACUCGGCUC	1576	1315	CCCAGCAUCUACUCGGCUC	1576	1337	GACGGAGUAGUGCUGGG	1823
1333	CACAGGCCAGGCCCUCA	1577	1333	CACAGGCCAGGCCCUCA	1577	1355	UGAGGGCCUGGGCUGUG	1824
1351	ACCUGCACGGCCUACGGG	1578	1351	ACCUGCACGGCCUACGGG	1578	1373	CCCCGUAGGGCUGCAGGU	1825
1369	GUGCCCCUGCCUCUAGCA	1579	1369	GUGCCCCUGCCUCUAGCA	1579	1391	UGCUGAGGGCAGGGGCAC	1826
1387	AUCCAGUGGCACUGGGCG	1580	1387	AUCCAGUGGCACUGGGCG	1580	1409	GCGGCCAGUGCCACUGGAU	1827
1405	CCCUGGACACCCUGCAAGA	1581	1405	CCCUGGACACCCUGCAAGA	1581	1427	UCUUGCAGGGUGUCCAGGG	1828
1423	AUGUUUCCCCAGCUGUAC	1582	1423	AUGUUUCCCCAGCUGUAC	1582	1445	GACUAGCUGGGCAACAU	1829
1441	CUCGGGGGGCAGCAGC	1583	1441	CUCGGGGGGCAGCAGC	1583	1463	GCUGCUGGCCGGGGAG	1830
1459	CAAGACCUCAUGCACAGU	1584	1459	CAAGACCUCAUGCACAGU	1584	1481	ACUGUGGCAUGGGGUUUG	1831
1477	UGCCGUGACUGGGCGG	1585	1477	UGCCGUGACUGGGCGG	1585	1499	CGGCCCUCCAGUACGGCA	1832
1495	GUGACCACGCCAGGAUGCC	1586	1495	GUGACCACGCCAGGAUGCC	1586	1517	GGGCAUCCUGGGGUUCAC	1833
1513	GUGAACCCCAUCGAGGCC	1587	1513	GUGAACCCCAUCGAGGCC	1587	1535	GGCUCUCGAUGGGGUUCAC	1834
1531	CUGGACACCCUGGACCGAGU	1588	1531	CUGGACACCCUGGACCGAGU	1588	1553	ACUCGGGUCCAGGGUCCAG	1835
1549	UUUUGGGAGGGAAAAGAAUA	1589	1549	UUUUGGGAGGGAAAAGAAUA	1589	1571	UAUUCUUUCCCCACACAA	1836

1567	AAGACUGUGAGGCAAGCUGG	1590	1567	AAGACUGUGAGGCAAGCUGG	1590	1589	CCAGCUUGCACAGCUU	1837
1585	GUGAUCCAGAAUUGCCAAACG	1591	1585	GUGAUCCAGAAUUGCCAAACG	1591	1607	CGUUGGCAUUCUGAUCAC	1838
1603	GUGUCUGGCCAUGUACAAAGU	1592	1603	GUGUCUGGCCAUGUACAAAGU	1592	1625	ACUUGUACAUGGCCAGACAC	1839
1621	UGUGUGGUCCUCAACAAAGG	1593	1621	UGUGUGGUCCUCAACAAAGG	1593	1643	CCUUGUJGGAGACACACAA	1840
1639	GUGGCCAGGAUGAGCGGC	1594	1639	GUGGCCAGGAUGAGCGGC	1594	1661	GCGCUCAUCCUGGCCAC	1841
1657	CUCAUCAUCUCAUGUGA	1595	1657	CUCAUCAUCUCAUGUGA	1595	1679	UCACAUAGAAGUAGAUGAG	1842
1675	ACCAACAUCCCCGACGGCU	1596	1675	ACCAACAUCCCCGACGGCU	1596	1697	AGCCGUCCCCGAAUGGUGGU	1843
1693	UUCACCAUCGAAUCCAAAGC	1597	1693	UUCACCAUCGAAUCCAAAGC	1597	1715	GUUUGGAAUUCGAUGGUGAA	1844
1711	CCAUCCGAGGGAGCUACUAG	1598	1711	CCAUCCGAGGGAGCUACUAG	1598	1733	CUAGUAGCUCCUCCGGAUGG	1845
1729	GAGGCCAGCCGGUGCUCC	1599	1729	GAGGCCAGCCGGUGCUCC	1599	1751	GGAGCACCCGGCUGGCCCCUC	1846
1747	CUGAGCUGCCAAAGCCGACA	1600	1747	CUGAGCUGCCAAAGCCGACA	1600	1769	UGUCGGGUUGGCAGCUAG	1847
1765	AGCUACAAAGUACGAGCAUC	1601	1765	AGCUACAAAGUACGAGCAUC	1601	1787	GAUGCUGUACUIGUAGCU	1848
1783	CUGGCGUGGUACCGCCCUA	1602	1783	CUGGCGUGGUACCGCCCUA	1602	1805	UGAGGGGUACCGCCAG	1849
1801	AACCUGUCACGGCUGCACG	1603	1801	AACCUGUCACGGCUGCACG	1603	1823	CGUGCGAGCGUGGACAGGUU	1850
1819	GAUGGCCACGGGAACCCGC	1604	1819	GAUGGCCACGGGAACCCGC	1604	1841	GCGGGGUUCCCGUGGCCAUC	1851
1837	CUUCUGCUCGACUGCAAGA	1605	1837	CUUCUGCUCGACUGCAAGA	1605	1859	UCUUGCAUGCAGCAGAAAG	1852
1855	AACGUGGAUCUGUUGCCA	1606	1855	AACGUGGAUCUGUUGCCA	1606	1877	UGGGGAACAGAUGCACGUU	1853
1873	ACCCCUCCUGGCCAGCC	1607	1873	ACCCCUCCUGGCCAGCC	1607	1895	GGCUGGGGCCAGAGGGGU	1854
1891	CUGGAGGGUGGGCACCUG	1608	1891	CUGGAGGGUGGGCACCUG	1608	1913	CAGGUGCCACCUCCUCCAG	1855
1909	GGGGCGCCACGCCACGC	1609	1909	GGGGCGCCACGCCACGC	1609	1931	GGGUGGGUGGGGCC	1856
1927	CUCAGCCUGAGUAUCCCC	1610	1927	CUCAGCCUGAGUAUCCCC	1610	1949	GGGGGAUACUCAGGCUGAG	1857
1945	CGCGUCGGCCGGAGCACG	1611	1945	CGCGUCGGCCGGAGCACG	1611	1967	CGUGCUUGGGGGGAGCGCG	1858
1963	GAAGGGCCACAUUGUGGCG	1612	1963	GAAGGGCCACAUUGUGGCG	1612	1985	CGCACACAUAGUGGCCUC	1859
1981	GAAGUGGAAGACGGCGCA	1613	1981	GAAGUGGAAGACGGCGCA	1613	2003	UGCGCCGGGUUCUAGGGCU	1860
1999	AGCCAUGACAAAGCACUGCC	1614	1999	AGCCAUGACAAAGCACUGCC	1614	2021	GGCAGUGGUUCUAGGGCU	1861
2017	CACAAAGAAGUACCUUCG	1615	2017	CACAAAGAAGUACCUUCG	1615	2039	CGGACAGGUACUUCUUGUG	1862
2035	GUGCAGGGCCUGGAAAGCCC	1616	2035	GUGCAGGGCCUGGAAAGCCC	1616	2057	GGGCUUCCAGGGCCUGCAC	1863
2053	CCUCGGCUACGCGAGACU	1617	2053	CCUCGGCUACGCGAGACU	1617	2075	AGUUUCUGCGUGGCCGAGG	1864
2071	UGACCCGACCUUCGUGA	1618	2071	UGACCCGACCUUCGUGA	1618	2093	UCACCAAGGGGUUCGGUAA	1865
2089	AACGUGAGGGACUCGUGG	1619	2089	AACGUGAGGGACUCGUGG	1619	2111	CCAGCGAGUGGCCUGACGUU	1866
2107	GAGAUGCGAGGUUGUGGG	1620	2107	GAGAUGCGAGGUUGUGGG	1620	2129	CCACCAAGCACUGCAUCUC	1867
2125	GCCGGAGGGCACGGCCCA	1621	2125	GCCGGAGGGCACGGCCCA	1621	2147	UGGGCGGGUGGCCUGGGC	1868
2143	AGCAUCUGUGGUACAAAG	1622	2143	AGCAUCUGUGGUACAAAG	1622	2165	CUUUGUACACAGGAUGGU	1869

2161	GACGAGAGGCUGGUGGAGG	1623	2161	GACCGAGGGCUGGUGGAGG	1623	2183	CCUCCAGGCAGCCUCUCGUC	1870
2179	AAAAAGUCUGGAGUGACU	1624	2179	GAAGAGUCUGGAGUGGACU	1624	2201	AGUCGACUCCAGACUUUUC	1871
2197	UUGGGGGACUCCAACCAGA	1625	2197	UUGGGGGACUCCAACCAGA	1625	2219	UCUGGGUGGAGUGGCCAA	1872
2215	AAGCUGAGCAUCCAGCGCG	1626	2215	AAGCUGAGCAUCCAGCGCG	1626	2237	CGCGCUGGAUGUCAGCUU	1873
2233	GUGCGCGAGGGAGGAUGCGG	1627	2233	GUGCGCGAGGGAGGAUGCGG	1627	2255	CGCAUCCUCUCGGGCAC	1874
2251	GGACCGUAUCUGUGCAGCG	1628	2251	GGACCGUAUCUGUGCAGCG	1628	2273	CGCUGCACAGAUACGGGUCC	1875
2269	GUGGCGAGCCAAAGGGCU	1629	2269	GUGGCGAGCCAAAGGGCU	1629	2291	AGCCCCUUGGGUCUGCACAC	1876
2287	UGCGUCAACCUCCGGCCA	1630	2287	UGCGUCAACCUCCGGCCA	1630	2309	UGGCGGAGGGAGGUACGCAC	1877
2305	AGCGUGGCCGUGGAAGGU	1631	2305	AGCGUGGCCGUGGAAGGU	1631	2327	AGCCUUCCACGGCACCGCU	1878
2323	UCCGAGGAUAGGGAGCA	1632	2323	UCCGAGGAUAGGGAGCA	1632	2345	UGCUGCCCCUUAUCUCUGGA	1879
2341	AUGGAGAUCUGGAUCCUUG	1633	2341	AUGGAGAUCUGGAUCCUUG	1633	2363	CAAGGAUCACGGAUCUCCAU	1880
2359	GUCCGUACCGGGGUCAUCG	1634	2359	GUCCGUACCGGGGUCAUCG	1634	2381	CGAUGACGCCGGUACCGAC	1881
2377	GCUGUCUUCUUCUGGUCC	1635	2377	GCUGUCUUCUUCUGGUCC	1635	2399	GGACCCAGAAAGAACAGC	1882
2395	CUCUCCUCCUCAUCUUCU	1636	2395	CUCUCCUCCUCAUCUUCU	1636	2417	AGAAGAUGAGGGAGGGAG	1883
2413	UGUAACAUAGGGAGGCCGG	1637	2413	UGUAACAUAGGGAGGCCGG	1637	2435	CGGGCCUCCUCAUGUUUACA	1884
2431	GCCCCACGCGAGACAUCAAGA	1638	2431	GCCCCACGCGAGACAUCAAGA	1638	2453	UCUUGAUAGUCUGGGGGC	1885
2449	ACGGGCUACCUUCGCAUCA	1639	2449	ACGGGCUACCUUCGCAUCA	1639	2471	UGAUGGGACAGGUAGGCCGU	1886
2467	AUCAUUGGACCCCCGGGAGG	1640	2467	AUCAUUGGACCCCCGGGAGG	1640	2489	CCUCCCCGGGGUCUCAUGAU	1887
2485	GUGCCUCUGGAGGAGCAAU	1641	2485	GUGCCUCUGGAGGAGCAAU	1641	2507	AUUGCCUCCUCCAGAGGCAC	1888
2503	UGCGAAUACCUGGUCCUACG	1642	2503	UGCGAAUACCUGGUCCUACG	1642	2525	CGUAGGGACAGGUAUUCGCA	1889
2521	GAUGCCAGCCAGUGGGAAU	1643	2521	GAUGCCAGCCAGUGGGAAU	1643	2543	AUUCCACUGGGUGGCAUC	1890
2539	UUCCCCCGGAGAGGGCUGC	1644	2539	UUCCCCCGGAGAGGGCUGC	1644	2561	GCAGCCGGCUCUCGGGGAA	1891
2557	CACCUUGGGAGAGGUCCUCG	1645	2557	CACCUUGGGAGAGGUCCUCG	1645	2579	CGAGCACUCUCCAGGGUG	1892
2575	GGCUACGGGCCUUUGGGA	1646	2575	GGCUACGGGCCUUUGGGA	1646	2597	UCCCGAAGGGGGGUAGGCC	1893
2593	AAGGUGGGUGGAAGGCCUCCG	1647	2593	AAGGUGGGUGGAAGGCCUCCG	1647	2615	CGGAGGGUUCUCCACCCUU	1894
2611	GCUUUUCGGCAUCCACAAGG	1648	2611	GCUUUUCGGCAUCCACAAGG	1648	2633	CCUUGUGGAUGGCCAAAGC	1895
2629	GGCAGCAGCUGUGACACCG	1649	2629	GGCAGCAGCUGUGACACCG	1649	2651	CGGUGUCACAGCUGCUGCC	1896
2647	GUGGCCUGAAAAUGCUGA	1650	2647	GUGGCCUGAAAAUGCUGA	1650	2669	UCAGCAUUUUUCAGGCCAC	1897
2665	AAAGAGGGCGCCACGGCCA	1651	2665	AAAGAGGGCGCCACGGCCA	1651	2687	UGGCCUGGGGCCUCUUU	1898
2683	AGCGAGGAGCGGCCUGA	1652	2683	AGCGAGGAGCGGCCUGA	1652	2705	UCAGCGCGGCCUGCCUGCU	1899
2701	AUGUCGGAGCUCAAGAUCC	1653	2701	AUGUCGGAGCUCAAGAUCC	1653	2723	GAUCUUGAGCUUCGACAU	1900
2719	CUCAUUCAUCAUCGGCAACC	1654	2719	CUCAUUCAUCAUCGGCAACC	1654	2741	GGUUGCCGAUGUGAAUGAG	1901
2737	CACCUUCAACGUGGUCAACC	1655	2737	CACCUUCAACGUGGUCAACC	1655	2759	GGUUGACCACGUUGGAGGUG	1902

2755	CUCCUCGGGGUGGCACCA	1656	2755	CUCCUCGGGGUGGCACCA	1656	2777	UGGUGGCACGGCCCCGAGGAG	1903
2773	AAGCCGCAGGGCCCCCUA	1657	2773	AAGCCGCAGGGCCCCCUA	1657	2795	UGAGGGGGCCUGGGCCUU	1904
2791	AUGGUGAUCCGGGAGUUCU	1658	2791	AUGGUGAUCCGGGAGUUCU	1658	2813	AGAACUCCACGAUCACCAU	1905
2809	UGCAAGUACGGCAACCUCU	1659	2809	UGCAAGUACGGCAACCUCU	1659	2831	AGAGGUUGGCCGUACUUGCA	1906
2827	UCCAACUUCUCCUGGGGCCA	1660	2827	UCCAACUUCUCCUGGGGCCA	1660	2849	UGGCGCCAGGAAGUUGGA	1907
2845	AAGGGGGAGCCUUCAGCC	1661	2845	AAGGGGGAGCCUUCAGCC	1661	2867	GGCUGAAGGGGUCCCCGUU	1908
2863	CCCUGCGGGAGAAGUCUC	1662	2863	CCCUGCGGGAGAAGUCUC	1662	2885	GAGACUUUCUCCGGCAGGG	1909
2881	CCCGAGGAGCGGGGACGCU	1663	2881	CCCGAGGAGCGGGGACGCU	1663	2903	AGCGUCCGGCUCGUCCGG	1910
2899	UUCCGGCCAUGGUUGGAGC	1664	2899	UUCCGGCCAUGGUUGGAGC	1664	2921	GUCCACCAUGGGGCCGGAA	1911
2917	CUCGCCAGGGCUGGAUCGGA	1665	2917	CUCGCCAGGGCUGGAUCGGA	1665	2939	UCCGAUCCAGGCCUGGGCAG	1912
2935	AGGGGGGGGGAGCAGCGG	1666	2935	AGGGGGGGGGAGCAGCGG	1666	2957	CGCUGCUCCCCGGGGCCU	1913
2953	GACAGGGGUCCUCUUCGGCG	1667	2953	GACAGGGGUCCUCUUCGGCG	1667	2975	GGCGGAAGGAGGCCUGUC	1914
2971	CGGUUCUCCGAAAGCCGAGG	1668	2971	CGGUUCUCCGAAAGCCGAGG	1668	2993	CCUCGGGUCCUUCGAGAACCG	1915
2989	GGCGGAGGGAGGGGGCUU	1669	2989	GGCGGAGGGAGGGGGCUU	1669	3011	AAGCCGCCUCCGGGGCC	1916
3007	UCUCCAGACCAAGAACGUG	1670	3007	UCUCCAGACCAAGAACGUG	1670	3029	CAGCUUCUCCGGUGGAGA	1917
3025	GAGGACCCUGGGCUGAGCC	1671	3025	GAGGACCCUGGGCUGAGCC	1671	3047	GGCUCAGCCACAGGUCCUC	1918
3043	CGGCUGACCAUGGAAGAU	1672	3043	CGGCUGACCAUGGAAGAU	1672	3065	GAUCUUCUCCAGGGCAG	1919
3061	CUUUCUGCUACAGCUUCC	1673	3061	CUUUCUGCUACAGCUUCC	1673	3083	GGAAAGCUUCCAGAACAG	1920
3079	CAAGGUGGCCAGGGGAUGG	1674	3079	CAAGGUGGCCAGGGGAUGG	1674	3101	CCAUCCUCCUGGGCACCUG	1921
3097	GAGUUCUCCGGCUUCCCGAA	1675	3097	GAGUUCUCCGGCUUCCCGAA	1675	3119	UUCGGGAAGGCCAGGAACUC	1922
3115	AAGUGCAUCCACAGAGACC	1676	3115	AAGUGCAUCCACAGAGACC	1676	3137	GGUCUUCUGGGGAUCACCU	1923
3133	CUGGCGCUCGGAAACAUUC	1677	3133	CUGGCGCUCGGAAACAUUC	1677	3155	GAAUGGUCCGAGCAGCCAG	1924
3151	CUGCGUGCGGGAAAGCGACG	1678	3151	CUGCGUGCGGGAAAGCGACG	1678	3173	CGUCGCUUUCGACAGCAG	1925
3169	GUGGUGAAGAUCUGUGACU	1679	3169	GUGGUGAAGAUCUGUGACU	1679	3191	AGUCACAGAACUUCACAC	1926
3187	UUUGGCCUUGCCGGGACA	1680	3187	UUUGGCCUUGCCGGGACA	1680	3209	UGUCCGGGCAAGGCCAAA	1927
3205	AUCUACAAAGACCCCGACU	1681	3205	AUCUACAAAGACCCCGACU	1681	3227	AGUCGGGGGUUUUUGUAGAU	1928
3223	UACGUCCGCAAGGGCAGUG	1682	3223	UACGUCCGCAAGGGCAGUG	1682	3245	CACUGCCCUUGCGGACGUA	1929
3241	GCCCCGGCUGCCCCUAGU	1683	3241	GCCCCGGCUGCCCCUAGU	1683	3263	ACUUUCAGGGGCCAGCCGGC	1930
3259	UGGAUGGGCCCCUGAAAGCA	1684	3259	UGGAUGGGCCCCUGAAAGCA	1684	3281	UGCUUUCAGGGCCAUCCA	1931
3277	AUCUUCGACAAGGGGUACA	1685	3277	AUCUUCGACAAGGGGUACA	1685	3299	UGUACACCUUCCUGGAAGAU	1932
3295	ACCAAGCGAGAGUGUGU	1686	3295	ACCAAGCGAGAGUGUGU	1686	3317	ACACGUACUCUGGGGUGGU	1933
3313	UGGUCCUUUGGGGUUCUUC	1687	3313	UGGUCCUUUGGGGUUCUUC	1687	3335	GAAGCACCCCAAGGGACCA	1934
3331	CUCUGGGAGAUCUUCUCUC	1688	3331	CUCUGGGAGAUCUUCUCUC	1688	3353	GAGAGAAGAUCUCCCAAGAG	1935

3349	CUGGGGCCUCCCGUACC	1689	3349	CUGGGGCCUCCCGUACC	1689	3371	GGUACGGGGAGGCCAG	1936
3367	CCUGGGGUGCGAGAUCAUG	1690	3367	CCUGGGGUGCGAGAUCAUG	1690	3389	CAUUGAUCUGCACCCAGG	1937
3385	GAGGAGUUCUGCCAGCGG	1691	3385	GAGGAGUUCUGCCAGCGG	1691	3407	CGCGCUGGGAGAACUCCUC	1938
3403	GUGAGAGACGGCACAGGA	1692	3403	GUGAGAGACGGCACAGGA	1692	3425	UCCUUGUGCCGUCUCUAC	1939
3421	AUGAGGGCCCCGGAGCUGG	1693	3421	AUGAGGGCCCCGGAGCUGG	1693	3443	CCAGCUCCGGGGCCUCAU	1940
3439	GCCACUCGGCCAUACGCC	1694	3439	GCCACUCGGCCAUACGCC	1694	3461	GGCGUAUGGGGGAGUGGC	1941
3457	CACAUCAUGCGAACUGCU	1695	3457	CACAUCAUGCGAACUGCU	1695	3479	AGCAGUUCAGCAUGAUGUG	1942
3475	UGGUCCGGAGACCCCAAGG	1696	3475	UGGUCCGGAGACCCCAAGG	1696	3497	CCUUGGGGUCCGGGACCA	1943
3493	GCGAGACCUUGCAUUCUCGG	1697	3493	GCGAGACCUUGCAUUCUCGG	1697	3515	CCGAGAAUGCAGGUUCUGCG	1944
3511	GACCUUGGGAGAACUCCUGG	1698	3511	GACCUUGGGAGAACUCCUGG	1698	3533	CCAGGAUCUCCACAGGGUC	1945
3529	GGGGACCUCGUCCAGGGCA	1699	3529	GGGGACCUCGUCCAGGGCA	1699	3551	UGCCCUGGGAGCGGUCCCC	1946
3547	AGGGGCCUGCAAGAGGAAG	1700	3547	AGGGGCCUGCAAGAGGAAG	1700	3569	CUUCCUCUUGCGAGCCCCU	1947
3565	GAGGAGGUUCUGCAUGGGCC	1701	3565	GAGGAGGUUCUGCAUGGGCC	1701	3587	GGGCCAUGGAGACCUCCUC	1948
3583	CCGGCGAOGCUCUAGAGCU	1702	3583	CCGGCGAOGCUCUAGAGCU	1702	3605	AGCUCUGAGGCGUGGCGGG	1949
3601	UCAGAAAGGGCAGCUUCU	1703	3601	UCAGAAAGGGCAGCUUCU	1703	3623	AGAAGCGUGCCUCUUCUGA	1950
3619	UCGCAGGUUCACCAGGG	1704	3619	UCGCAGGUUCACCAGGG	1704	3641	CCAUGGGUGGACACUGCGA	1951
3637	GCCCCUACACAUUCGCCAGG	1705	3637	GCCCCUACACAUUCGCCAGG	1705	3659	CCUGGGCGAUGGUAGGGC	1952
3655	GCUGACGCCUGGAGCAGCC	1706	3655	GCUGACGCCUGGAGCAGCC	1706	3677	GGCUGGUCCUCAGCGUCAGC	1953
3673	CCGCCCAAGCCUGCGGCC	1707	3673	CCGCCCAAGCCUGCGGCC	1707	3695	GGCGCUGCGAGGCUUGGGGG	1954
3691	CACAGCCUGGCCAGGU	1708	3691	CACAGCCUGGCCAGGU	1708	3713	ACCUUGGGGGCCAGGGCUGUG	1955
3709	UAUUACAAUCUGGGGUCCU	1709	3709	UAUUACAAUCUGGGGUCCU	1709	3731	AGGACACCCAGGUUAUA	1956
3727	UUIUCGGGGUGCCUGGCCA	1710	3727	UUIUCGGGGUGCCUGGCCA	1710	3749	UGGCCAGGGCACCCGGAAA	1957
3745	AGAGGGGGUGAGACCCUG	1711	3745	AGAGGGGGUGAGACCCUG	1711	3767	CACGGGUUCUAGGCCCUU	1958
3763	GGUUCUCCAGGGAUGAAGA	1712	3763	GGUUCUCCAGGGAUGAAGA	1712	3785	UCUUCAUCUGGGAGGAACC	1959
3781	ACAUUUUGGGAAUUCCCA	1713	3781	ACAUUUUGGGAAUUCCCA	1713	3803	UGGGAAUUCUCAAAUGU	1960
3799	AUGACCCCAACGACCUACA	1714	3799	AUGACCCCAACGACCUACA	1714	3821	UGUAGGUUCUUGGGGUCAU	1961
3817	AAAGGGCUUGGGACAACC	1715	3817	AAAGGGCUUGGGACAACC	1715	3839	GGUUGGUCCACAGGCCUU	1962
3835	CAGACAGACAGUGGAUGG	1716	3835	CAGACAGACAGUGGAUGG	1716	3857	CCAUCCCACUGUGGUUC	1963
3853	GUUGCUUGGCCUCGGAGGU	1717	3853	GUUGCUUGGCCUCGGAGGU	1717	3875	ACUCCUCGAGGCCAGCAC	1964
3871	UUUGAGGAGAUAGAGAGCA	1718	3871	UUUGAGGAGAUAGAGAGCA	1718	3893	UGCUCUCUACUGGUCAAAA	1965
3889	AGGCAUAGACAAGAACCG	1719	3889	AGGCAUAGACAAGAACCG	1719	3911	CGGUUUUCUUGGUCAUGCCU	1966
3907	GGCUUUCAGGUAGCUGAAGC	1720	3907	GGCUUUCAGGUAGCUGAAGC	1720	3929	GCUUCAUCGUAGGAAGGCC	1967
3925	CAGAGGAGGAGAAGGCCAGC	1721	3925	CAGAGGAGGAGAAGGCCAGC	1721	3947	GCUGCCUUUCUUCUCUCUG	1968

3943	CAUACGUAGCAUUIUCU	1722	3943	CAUACGUAGCAUUIUCU	1722	3965	AAGAAAAUGCUGACGUAG	1969
3961	UCUCUGCACUUUAAGAAA	1723	3961	UCUCUGCACUUUAAGAAA	1723	3983	UUUCUUUAAGUGCAGAGA	1970
3979	AGAUCAAAGACUUUAAGAC	1724	3979	AGAUCAAAGACUUUAAGAC	1724	4001	GUUUAAAUGCUUUGAUU	1971
3997	CUUUCGUUUUCUUCUAC	1725	3997	CUUUCGUUUUCUUCUAC	1725	4019	GUAGAAAGAAAUAUGCGAAAG	1972
4015	CUGCUAUCUACUACAAACU	1726	4015	CUGCUAUCUACUACAAACU	1726	4037	AGUUUGUAGUAGUAGCAG	1973
4033	UUCAAAGAGGAACCAGGG	1727	4033	UUCAAAGAGGAACCAGGG	1727	4055	CUCCUGGUUCCUUUGAA	1974
4051	GGACAAGAGGGCAUGAAA	1728	4051	GGACAAGAGGGCAUGAAA	1728	4073	UUUCAUGCUCCUUGUCC	1975
4069	AGUGGACAAGGGAGUGAC	1729	4069	AGUGGACAAGGGAGUGAC	1729	4091	GUACACACUCCUUGUCCACU	1976
4087	CCACUGAAAGCACCACAGGG	1730	4087	CCACUGAAAGCACCACAGGG	1730	4109	CCUGUGGGGUUCCAGUGG	1977
4105	GAGGGGUJAGGCCUCGGGA	1731	4105	GAGGGGUJAGGCCUCGGGA	1731	4127	UCCGGAGGGCUAACCCUC	1978
4123	AUGACUGGGGGCAGGCCUG	1732	4123	AUGACUGGGGGCAGGCCUG	1732	4145	CAGGCCUGCCGGAGUCAU	1979
4141	GGAUAAAUAUCCAGCCUCCC	1733	4141	GGAUAAAUAUCCAGCCUCCC	1733	4163	GGGAGGCUGGAAUUAUCC	1980
4159	CACAAAGAAGCUGGUGGAGC	1734	4159	CACAAAGAAGCUGGUGGAGC	1734	4181	GUCCACCCAGCUUCCUUGUG	1981
4177	CAGAGUGUUCCUGACUCC	1735	4177	CAGAGUGUUCCUGACUCC	1735	4199	GGAGUCAGGGAACACUCUG	1982
4195	CUCCAAGAAAAGGGAGACG	1736	4195	CUCCAAGAAAAGGGAGACG	1736	4217	CGUCUCUUUUCCUUGGAG	1983
4213	GCCCCUUUCAUGGUUCUGCUG	1737	4213	GCCCCUUUCAUGGUUCUGCUG	1737	4235	CAGCGAACCAUGAAAGGGC	1984
4231	GAGUAACAGGGGUUCCCC	1738	4231	GAGUAACAGGGGUUCCCC	1738	4253	GGGAAGGCACCUGUUACUC	1985
4249	CAGACACUGGGCUUACUGC	1739	4249	CAGACACUGGGCUUACUGC	1739	4271	GCAGUAACGCCAGUGUCUG	1986
4267	CUUGACCAAAGAGCCCUCA	1740	4267	CUUGACCAAAGAGCCCUCA	1740	4289	UGAGGGCUCUUUGGUCAAG	1987
4285	AAGCGGGCCUUAGGCCAGC	1741	4285	AAGCGGGCCUUAGGCCAGC	1741	4307	GCUGGCAUAGGGCCGUU	1988
4303	CGUGACAGAGGGCUACCU	1742	4303	CGUGACAGAGGGCUACCU	1742	4325	AGGUGAGGCCUCUGUCACG	1989
4321	UCUUGCCUUUCUAGGUACU	1743	4321	UCUUGCCUUUCUAGGUACU	1743	4343	AGUGACCUAGAAGCAAGA	1990
4339	UUCUCACAAUGUCCUUCA	1744	4339	UUCUCACAAUGUCCUUCA	1744	4361	UGAAGGGACAUUGUGAGAA	1991
4357	AGCACCUGACCCUGGGCCC	1745	4357	AGCACCUGACCCUGGGCCC	1745	4379	GGGCACAGGGUCAGGGGU	1992
4375	CGCCGAUUAUCCUUGGU	1746	4375	CGCCGAUUAUCCUUGGU	1746	4397	UACCAAGGAAUACGGGCG	1993
4393	AAUUAUGAGUAAUACUCAA	1747	4393	AAUUAUGAGUAAUACUCAA	1747	4415	UUGAUGUAAUACUAAU	1994
4411	AAGAGUAGUAAUAAAAGCU	1748	4411	AAGAGUAGUAAUAAAAGCU	1748	4433	AGCUUUUUACUACUCUU	1995
4429	UAAAUAUCAUGUUUAUA	1749	4429	UAAAUAUCAUGUUUAUA	1749	4451	UUAUAAAACAUGAUUAUA	1996

The 3'-ends of the Upper sequence and the Lower sequence of the siNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complementary to a portion of the target sequence. The overhang can comprise the general

structure NN or NsN, where N stands for any nucleotide (e.g., thymidine) and s stands for phosphorothioate or other internucleotide linkage as described herein (e.g. internucleotide linkage having Formula I). The upper sequence is also referred to as the sense strand, whereas the lower sequence is also referred to as the antisense strand. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII or any combination thereof (see for example chemical modifications as shown in Table V herein).

Table III: VEGFr Synthetic Modified siNA constructs

VEGFR1

Target	Seq ID	COMPOUND#	Aliases	Sequence	Seq ID
GCUGUCUGCUUCUCACAGGAUCU	1997		FLT1:298U21 siRNA sense	UGUCUGCUUCUCACAGGAU TT	2020
GAAGGAGGACCUGAAACUGUC	1998		FLT1:1956U21 siRNA sense	AGGAGAGGACCUGAAACUGTT	2021
AAGGAGGACCUGAAACUGUC	1999		FLT1:1957U21 siRNA sense	GGAGAGGACCUGAAACUGUTT	2022
GCAUUJGGCAUUAAGAAUCACC	2000		FLT1:2787U21 siRNA sense	AUUGGCAUUAAGAAUCATT	2023
GCUGUCUGCUUCUCACAGGAUCU	1997		FLT1:316L21 siRNA (298C) antisense	AUCCUGUGAGAAGCAGACATT	2024
GAAGGAGGACCUGAAACUGUC	1998		FLT1:1974L21 siRNA (1956C) antisense	CAGUUUCAGGUCCUCUCUTT	2025
AAGGAGGACCUGAAACUGUC	1999		FLT1:1975L21 siRNA (1957C) antisense	ACAGUUUCAGGUCCUCUCCTT	2026
GCAUUJGGCAUUAAGAAUCACC	2000		FLT1:2805L21 siRNA (2787C) antisense	UGAUUUUCUUAUGCCAAAU TT	2027
GCUGUCUGCUUCUCACAGGAUCU	1997		FLT1:298U21 siRNA stab04 sense	B uGcuGcuucucAGGAU TT B	2028
GAAGGAGGACCUGAAACUGUC	1998		FLT1:1956U21 siRNA stab04 sense	B AGGAGAGGACCUGAAACuGTT B	2029
AAGGAGGACCUGAAACUGUC	1999		FLT1:1957U21 siRNA stab04 sense	B GGAGAGGACCUGAAACuGUTT B	2030
GCAUUJGGCAUUAAGAAUCACC	2000		FLT1:2787U21 siRNA stab04 sense	B AuuGGGAAuUAAGAAACuATT B	2031
GCUGUCUGCUUCUCACAGGAUCU	1997		FLT1:316L21 siRNA (298C) stab05 antisense	AuccuGUAGAGAA GcAGAcATsT	2032
GAAGGAGGACCUGAAACUGUC	1998		FLT1:1974L21 siRNA (1956C) stab05 antisense	cAGuucAGGGuccucuccuTsT	2033
AAGGAGGACCUGAAACUGUC	1999		FLT1:1975L21 siRNA (1957C) stab05 antisense	AcAGuuuAGGGuuccuccTsT	2034
GCAUUJGGCAUUAAGAAUCACC	2000		FLT1:2805L21 siRNA (2787C) stab05 antisense	uGAuuucuuAAuGccAAuTsT	2035
GCUGUCUGCUUCUCACAGGAUCU	1997		FLT1:298U21 siRNA stab07 sense	B uGcuGcuucucAGGAU TT B	2036
GAAGGAGGACCUGAAACUGUC	1998		FLT1:1956U21 siRNA stab07 sense	B AGGAGAGGACCUGAAACuGTT B	2037
AAGGAGGACCUGAAACUGUC	1999		FLT1:1957U21 siRNA stab07 sense	B GGAGAGGACCUGAAACuGUTT B	2038
GCAUUJGGCAUUAAGAAUCACC	2000		FLT1:2787U21 siRNA stab07 sense	B AuuGGGAAuUAAGAAACuATT B	2039
GCUGUCUGCUUCUCACAGGAUCU	1997		FLT1:316L21 siRNA (298C) stab11 antisense	AuccuGUAGAGAA GcAGAcATsT	2040
GAAGGAGGACCUGAAACUGUC	1998		FLT1:1974L21 siRNA (1956C) stab11 antisense	cAGuucAGGGuccucuccuTsT	2041
AAGGAGGACCUGAAACUGUC	1999		FLT1:1975L21 siRNA (1957C) stab11 antisense	AcAGuuuAGGGuuccuccTsT	2042
GCAUUJGGCAUUAAGAAUCACC	2000		FLT1:2805L21 siRNA (2787C) stab11 antisense	uGAuuucuuAAuGccAAuTsT	2043
AACUGAGUUAAAAGGCACCCAG	2009	31209	FLT1:367L21 siRNA (349C) stab05 inv antisense	GAucuAAuuiuucGuGGGTsT	2176
AAGCAAGGGGGCCUCUGAUGGU	2012	31210	FLT1:2967L21 siRNA (2949C) stab05 inv antisense	cGuuccuccGGAGAcuActTsT	2177

AGCCUGGAAAGAAUCAAAACCUU	2011	31211	FLT1:3930L21 siRNA (3912C) stab05 inv antisense	GGAccuuuuuuuAGuuuuGGTst	2178
AACUGAGUUUUAAAAGGCACCCAG	2009	31212	FLT1:349U21 siRNA stab07 inv sense	B cccAcGGAAAAGuuuGAGGucTT B	2179
AAGCAAGGAGGGCCUCUGAUGGU	2012	31213	FLT1:2949U21 siRNA stab07 inv sense	B GUAGucuccGGGAGGAACGTT B	2180
AGCCUGGAAAGAAUCAAAACCUU	2011	31214	FLT1:3912U21 siRNA stab07 inv sense	B ccAAAAACuAAAGAAAGGucTT B	2181
AACUGAGUUUUAAAAGGCACCCAG	2009	31215	FLT1:367L21 siRNA (349C) stab08 inv antisense	GAcuCAAAuuumcGuGGGtst	2182
AAGCAAGGAGGGCCUCUGAUGGU	2012	31216	FLT1:2967L21 siRNA (2949C) stab08 inv antisense	cGuuuccuuccGGAGAcuActst	2183
AGCCUGGAAAGAAUCAAAACCUU	2011	31217	FLT1:3930L21 siRNA (3912C) stab08 inv antisense	GGAccuuuuuuuAGuuuuGGTst	2184
AACUGAGUUUUAAAAGGCACCCAG	2009	31270	FLT1:349U21 siRNA stab09 sense	B CUGAGUUUAAAAGGCAACCTT B	2185
AAGCAAGGAGGGCCUCUGAUGGU	2012	31271	FLT1:2949U21 siRNA stab09 sense	B GCAAGGAGGGCCUCUGAUGTT B	2186
AGCCUGGAAAGAAUCAAAACCUU	2011	31272	FLT1:3912U21 siRNA stab09 sense	B CCUGAAAAGAAUCAAAACCTT B	2187
AACUGAGUUUUAAAAGGCACCCAG	2009	31273	FLT1:367L21 siRNA (349C) stab10 antisense	GGGUGCCUUUUAAACUCAGTst	2188
AAGCAAGGAGGGCCUCUGAUGGU	2012	31274	FLT1:2967L21 siRNA (2949C) stab10 antisense	CAUCAGAGGCCCUUUGCTst	2189
AGCCUGGAAAGAAUCAAAACCUU	2011	31275	FLT1:3930L21 siRNA (3912C) stab10 antisense	GGUUUJGAUuUuUCCAGGtst	2190
AACUGAGUUUUAAAAGGCACCCAG	2009	31276	FLT1:349U21 siRNA stab09 inv sense	B CCCACGGAAAAUUUUGAGUCTT B	2191
AAGCAAGGAGGGCCUCUGAUGGU	2012	31277	FLT1:2949U21 siRNA stab09 inv sense	B GUAGUCUCCGGGAGGAACGTT B	2192
AGCCUGGAAAGAAUCAAAACCUU	2011	31278	FLT1:3912U21 siRNA stab09 inv sense	B CCAAAACUAAGGAAAGGUCCTT B	2193
AACUGAGUUUUAAAAGGCACCCAG	2009	31279	FLT1:367L21 siRNA (349C) stab10 inv antisense	GACUCAAAUUUUCCGGGAGACtst	2194
AAGCAAGGAGGGCCUCUGAUGGU	2012	31280	FLT1:2967L21 siRNA (2949C) stab10 inv antisense	CGUICCUCCGGGAGACtst	2195
AGCCUGGAAAGAAUCAAAACCUU	2011	31281	FLT1:3930L21 siRNA (3912C) stab10 inv antisense	GGACCUUUUCUJAGUUUUUGGtst	2196
AACAACCAACAAAUAACAAGA	2010	31424	BDu antisense	uuGuGuAuuuuGuGGGuuGxst	2197
AAGCAAGGAGGGCCUCUGAUGGU	2012	31425	BDu antisense	cAucAGGGGccuccuuGcxst	2198
AACAACCAACAAAUAACAAGA	2010	31442	BDu antisense	uuGuGuAuuuuGuGGGuuGxst	2199
AAGCAAGGAGGGCCUCUGAUGGU	2012	31443	BDu antisense	cAucAGGGGccuccuuGcxst	2200
AACAACCAACAAAUAACAAGA	2010	31449	FLT1:2358L21 siRNA (2340C) stab11 3'-	B CAACCCACAAAUAACAACTT B	2201
AACAACCAACAAAUAACAAGA	2010	31450	BDu antisense	B AACAAACAAAACACCAACTT B	2202
AACAACCAACAAAUAACAAGA	2010	31451	FLT1:2358L21 siRNA (2340C) stab10 antisense	UUGUUGUAUUUUUGGGUUGGtst	2203
AACAACCAACAAAUAACAAGA	2010	31452	FLT1:2358L21 siRNA (2340C) inv stab10 antisense	GUUGGUGUUUAUGUUUUTst	2204

AACAAACCACAAUACAAAGA	2010	31509	FLT1:2358L21 siRNA (2340C) stab11 antisense	uuGuuGuAuuuuGuGGuuGtst	2217
AACUGAGUUAAAAGGCAACCGAG	2009	31794	2x cholesterol + R31194 FLT1:349U21 siRNA stab07 sense	(H)2 ZTa B cuGAGuuAAAAGGcAccCTT B	2218
AACUGAGUUAAAAGGCAACCGAG	2009	31795	2x cholesterol + R31212 FLT1:349U21 siRNA stab07 inv sense	(H)2 ZTa B cccAccGGAAAAuuuGAGucTT B	2219
AACUGAGUUAAAAGGCAACCGAG	2009	31796	2x cholesterol + R31270 FLT1:349U21 siRNA stab09 sense	(H)2 ZTa B CUGAGUUAAAAGGcAccCTT B	2220
AACUGAGUUAAAAGGCAACCGAG	2009	31797	2x cholesterol + R31276 FLT1:349U21 siRNA stab09 inv sense	(H)2 ZTa B CCCACGGAAAuuuJUGAGucTT B	2221
AACUGAGUUAAAAGGCAACCGAG	2009	31798	2x C18 phospholipid + R31194 FLT1:349U21 siRNA stab07 sense	(L)2 ZTa B cuGAGuuAAAAGGcAccCTT B	2222
AACUGAGUUAAAAGGCAACCGAG	2009	31799	2x C18 phospholipid + R31212 FLT1:349U21 siRNA stab07 inv sense	(L)2 ZTa B cccAccGGAAAuuuGAGucTT B	2223
AACUGAGUUAAAAGGCAACCGAG	2009	31800	2x C18 phospholipid + R31270 FLT1:349U21 siRNA stab09 sense	(L)2 ZTa B CUGAGUUAAAAGGcAccCTT B	2224
AACUGAGUUAAAAGGCAACCGAG	2009	31801	2x C18 phospholipid + R31276 FLT1:349U21 siRNA stab09 inv sense	(L)2 ZTa B CCCACGGAAAuuuJUGAGucTT B	2225
CAUGCUGGACUGCUGGGCAC	2244	32235	FLT1:3645U21 siRNA sense	CAUGCUGGACUGCUGGGCAC	2275
AUGCUGGACUGCUGGGCAC	2245	32236	FLT1:3646U21 siRNA sense	AUGCUGGACUGCUGGGCAC	2276
UGCUGGACUGCUGGGCAC	2246	32237	FLT1:3647U21 siRNA sense	UGCUGGACUGCUGGGCACAGTT	2277
CAUGCUGGACUGCUGGGCAC	2244	32250	FLT1:3663U21 siRNA (3645C) antisense	GUGCCAGCAGUCCAGCAUGTT	2278
AUGCUGGACUGCUGGGCAC	2245	32251	FLT1:3664U21 siRNA (3646C) antisense	UGUGCCAGCAGUCCAGCAUUT	2279
UGCUGGACUGCUGGGCAC	2246	32252	FLT1:3665U21 siRNA (3647C) antisense	CUGUGGCCAGCAGUCCAGCATT	2280
AACUGAGUUAAAAGGCAACCGAG	2009	32278	FLT1:349U21 siRNA stab16 sense	B CUGAGUUAAAAGGcAccCTT B	2281
AACUGAGUUAAAAGGCAACCGAG	2009	32279	FLT1:349U21 siRNA stab18 sense	B cuGAGuuAAAAGGcAccCTT B	2282
AACUGAGUUAAAAGGCAACCGAG	2009	32280	FLT1:349U21 siRNA inv stab16 sense	B CCCaCggaaaauuuJugucTT B	2283
AACUGAGUUAAAAGGCAACCGAG	2009	32281	FLT1:349U21 siRNA inv stab18 sense	B cccAcGGAAAAuuuGAGucTT B	2284
CUGAACUGAGUUAAAAGGcACC	2247	32282	FLT1:346U21 siRNA stab09 sense	B GAAcUGAGUUAAAAGGcAccTT B	2285
UGAACUGAGUUAAAAGGcACC	2248	32283	FLT1:347U21 siRNA stab09 sense	B AACUGAGUUAAAAGGcAccTT B	2286
GAACUGAGUUAAAAGGcACC	2249	32284	FLT1:348U21 siRNA stab09 sense	B ACUGAGUUAAAAGGcAccTT B	2287
ACUGAGUUAAAAGGCAACCGAG	2250	32285	FLT1:350U21 siRNA stab09 sense	B UGAGUUAAAAGGcAccCCAGTT B	2288
CUGAGUUAAAAGGCAACCGAG	2251	32286	FLT1:351U21 siRNA stab09 sense	B GAGUUAAAAGGcAccCCAGTT B	2289
UGAGUUAAAAGGCAACCGAC	2252	32287	FLT1:352U21 siRNA stab09 sense	B AGUUAAAAGGcAccCCAGCTT B	2290
GAGUUAAAAGGCAACCGAC	2253	32288	FLT1:353U21 siRNA stab09 sense	B GUUUAAAAGGcAccCCAGCTT B	2291
CUGAACUGAGUUAAAAGGcACC	2247	32289	FLT1:364L21 siRNA (346C) stab10 antisense	UGCCUUUUAAAACUCAGUUCtst	2292
UGAACUGAGUUAAAAGGcACC	2248	32290	FLT1:365L21 siRNA (347C) stab10 antisense	UGGCCUUUUAAAACUCAGUUTst	2293
GAACUGAGUUAAAAGGCAACCA	2249	32291	FLT1:366L21 siRNA (348C) stab10 antisense	GGUGCCUUUUAAAACUCAGUTst	2294

ACUGAGUUAAAAGGCACCCAGC	2250	32292	FLT1:368L21 siRNA (350C) stab10 antisense	UGGGUGGCCUUUUAAACUCATsT	2295
CUGAGUUAAAAGGCACCCAGCA	2251	32293	FLT1:369L21 siRNA (351C) stab10 antisense	CUGGGUGGCCUUUUAAACUCTsT	2296
UGAGUUAAAAGGCACCCAGCAC	2252	32294	FLT1:370L21 siRNA (352C) stab10 antisense	GCUGGGUGGCCUUUUAAACUTsT	2297
GAGUUAAAAGGCACCCAGCAC	2253	32295	FLT1:371L21 siRNA (353C) stab10 antisense	UGCUUUGGGUGGCCUUUUAAACTsT	2298
CUGAACUGUUAAAAGGCACC	2247	32296	FLT1:346U21 siRNA inv stab09 sense	BACGGAAAAAUUUGAGUCAAGTtB	2299
UGAACUGGUUUAAAAGGCACCC	2248	32297	FLT1:347U21 siRNA inv stab09 sense	BCACGGAAAAAUUUGAGUCAATTB	2300
GAACUGAGUUAAAAGGCACCC	2249	32298	FLT1:348U21 siRNA inv stab09 sense	BCCACGGAAAAAUUUGAGUCATTB	2301
ACUGAGUUAAAAGGCACCCAGC	2250	32299	FLT1:350U21 siRNA inv stab09 sense	BACCCACGGAAAAAUUUGAGUTtB	2302
CUGAGUUAAAAGGCACCCAGCA	2251	32300	FLT1:351U21 siRNA inv stab09 sense	BGACCCACGGAAAAAUUUGAGTTB	2303
UGAGUUAAAAGGCACCCAGCAC	2252	32301	FLT1:352U21 siRNA inv stab09 sense	BCGACCCACGGAAAAAUUUGATTB	2304
GAGUUAAAAGGCACCCAGCAC	2253	32302	FLT1:353U21 siRNA inv stab09 sense	BAGACCCACGGAAAAAUUUGUTtB	2305
CUGAACUGGUUUAAAAGGCACC	2247	32303	FLT1:364L21 siRNA (346C) inv stab10 antisense	CUUGACUCAAAUUUUUCGUUTsT	2306
UGAACUGGUUUAAAAGGCACCC	2248	32304	FLT1:365L21 siRNA (347C) inv stab10 antisense	UUGACUCAAAUUUUUCGUUTsT	2307
GAACUGAGUUAAAAGGCACCC	2249	32305	FLT1:366L21 siRNA (348C) inv stab10 antisense	UGACUCAAAUUUUCGUUGGTsT	2308
ACUGAGUUAAAAGGCACCCAGC	2250	32306	FLT1:368L21 siRNA (350C) inv stab10 antisense	ACUCAAAUUUUUCGUUGGGUTsT	2309
CUGAGUUAAAAGGCACCCAGCA	2251	32307	FLT1:369L21 siRNA (351C) inv stab10 antisense	CUCAAAUUUUCGUUGGGUUCTsT	2310
UGAGUUAAAAGGCACCCAGCAC	2252	32308	FLT1:370L21 siRNA (352C) inv stab10 antisense	UCAAUAUUUUUCGUUGGGUCGTsT	2311
GAGUUAAAAGGCACCCAGCAC	2253	32309	FLT1:371L21 siRNA (353C) inv stab10 antisense	CAAAAUUUUCGUUGGGUGUTsT	2312
AACUGAGUUAAAAGGCACCCAG	2009	32338	FLT1:367L21 siRNA (349C) stab10 3'-BrdU antisense	GGGUGGCCUUUUAAACUCAGXsT	2313
AACUGAGUUAAAAGGCACCCAG	2009	32718	FLT1:367L21 siRNA (349C) v1 5p antisense	pGGGUGGCCUUUUAAACUCAG	2314
AACUGAGUUAAAAGGCACCCAG	2009	32719	FLT1:367L21 siRNA (349C) v2 5p antisense	GAGUUAAAAGB	2315
AAGCAAGGAGGGCUCUGAUGGU	2012	32720	FLT1:2967L21 siRNA (2949C) v1 5p antisense	pCAUCAGAGGCCCUUUGC	2316
AAGCAAGGAGGGCUCUGAUGGU	2012	32721	FLT1:2967L21 siRNA (2949C) v2 5p antisense	AAGGAGGGCCUCUGB	2317
AAGCAAGGAGGGCUCUGAUGGU	2012	32722	FLT1:2967L21 siRNA (2949C) v3 5p antisense	pCAUCAGAGGCCCUU	2318
CUGAACUGGUUUAAAAGGCACC	2247	32748	FLT1:346U21 siRNA stab07 sense	BGAACuGAGUUAAAAGGcATTB	2319
UGAACUGGUUUAAAAGGCACCC	2248	32749	FLT1:347U21 siRNA stab07 sense	BAAcuGAGUUAAAAGGcAcCTB	2320

AGTTAAAGGCACCCAGCACATC	2254	32806	FLT1:373L21 siRNA (354C) v2 5p antisense	pGUGCUGGGUGCCUUUAAA GGCACCCAGC B	2348
AGTTAAAGGCACCCAGCACATC	2254	32807	FLT1:373L21 siRNA (354C) v3 5p antisense	pGUGCUGGGUGCCUUUAGGCACCCAGC B	2349
GCATATATGATAAAGCATTCA	2255	32808	FLT1:1247L21 siRNA (1229C) v1 5p antisense	pAAUGCUUUAUCUAUAUAU	2350
GCATATATGATAAAGCATTCA	2255	32809	FLT1:1247L21 siRNA (1229C) v2 5p antisense	pAAUGCUUUAUCUAUAUAU	2351
GCATATATGATAAAGCATTCA	2255	32810	FLT1:1247L21 siRNA (1229C) v3 5p antisense	pAAUGCUUUAUCUAUAU	2352
GCATATATGATAAAGCATTCA	2255	32811	FLT1:1247L21 siRNA (1229C) v4 5p antisense	pAAUGCUUUAUCUAUAU	2353
GCATATATGATAAAGCATTCA	2255	32812	FLT1:1247L21 siRNA (1229C) v5 5p antisense	pAAUGCUUUAUCUAUAU	2354
GCATATATGATAAAGCATTCA	2255	32813	FLT1:1247L21 siRNA (1229C) v6 5p antisense	pAAUGCUUUAUCUAUAU	2355
AACUGAGUUAAAAGGCACCCAG	2009	33056	FLT1:367L21 siRNA (349C) v3 5p antisense	pGGGUGCCUUUAAAACUCAG GAGUUAAAAGG B	2356
AACUGAGUUAAAAGGCACCCAG	2009	33057	FLT1:367L21 siRNA (349C) v4 5p antisense	pGGGUGCCUUUAAAACUC GAGUUAAAAGGCA B	2357
AACUGAGUUAAAAGGCACCCAG	2009	33058	FLT1:367L21 siRNA (349C) v5 5p antisense	pGGGUGCCUUUAAAACU AGUUAAAAGG B	2358
AACUGAGUUAAAAGGCACCCAG	2009	33059	FLT1:367L21 siRNA (349C) v6 5p antisense	pGGGUGCCUUUAAAACU AGUUAAAAGG B	2359
AACUGAGUUAAAAGGCACCCAG	2009	33060	FLT1:367L21 siRNA (349C) v7 5p antisense	pGGGUGCCUUUAAAACU AGUUAAAAGGCA B	2360
AACUGAGUUAAAAGGCACCCAG	2009	33061	FLT1:367L21 siRNA (349C) v8 5p antisense	pGGGUGCCUUUAAAACU AGUUAAAAGGCA B	2361
AACUGAGUUAAAAGGCACCCAG	2009	33062	FLT1:367L21 siRNA (349C) v9 5p antisense	pGGGUGCCUUUAAAAC GUUUAAAAGG B	2362
AACUGAGUUAAAAGGCACCCAG	2009	33063	FLT1:367L21 siRNA (349C) v10 5p antisense	pGGGUGCCUUUAAAAC GUUUAAAAGGCA B	2363
AACUGAGUUAAAAGGCACCCAG	2009	33064	FLT1:367L21 siRNA (349C) v11 5p antisense	pGGGUGCCUUUAAAAC GUUUAAAAGGCA B	2364

VEGFR2

Target	Seq ID	COMPOUND#	Aliases	Sequence	Seq ID
UGACCUUGGAGCAUCUCAUCUGU	2001	KDR:3304U21	siRNA stab04 sense	B AccuGGAGCAucuAucuTT B	2052
UCACCUUUCCUGUAUGGAGGA	2003	KDR:3894U21	siRNA stab04 sense	B AccuGuuuccuGuAuGGAGTT B	2054
UGACCUUGGAGCAUCUCAUCUGU	2001	KDR:3322L21	siRNA (3304C) stab05 antisense	AGAUAGAGAUGuuccAAGGUTsT	2056
UCACCUUUCCUGUAUGGAGGA	2003	KDR:3912L21	siRNA (3894C) stab05 antisense	cucucauAcAGGAAAcAGGUUTsT	2058

UGACCUUUGGAGCAUCUCAUCUGU	2001	KDR:3304U21 siRNA stab07 sense	B AccuGGAGcAucuAucuTT B	2060
UCACCUUUCUCCUGUAUGGGAA	2003	32766 KDR:3894U21 siRNA stab07 sense	B AccuGuuuccuGuAuGGAGTT B	2062
UGACCUUUGGAGCAUCUCAUCUGU	2001	KDR:3322U21 siRNA (3304C) stab11 antisense	AGAuGAGAUGuccAAAGGUtSt	2064
UUUGAGCAUGGAAGGAAUCUG	2002	KDR:3872U21 siRNA (3894C) stab11 antisense	GAUuccuucuCCAuGcucATsT	2065
UCACCUUUCUCCUGUAUGGGAGGA	2003	KDR:3912U21 siRNA (3894C) stab11 antisense	cuccAuAcAGGAAACAGGUtSt	2066
GACAACACAGCAGGAAUCAGUCA	2004	KDR:3966U21 siRNA (3948C) stab11 antisense	AcuGAuuccuGcuGuGuuGtSt	2067
UGUCCACUUAUCCUGAGGAGCAAG	2017	30785 KDR:3076U21 siRNA stab04 sense	B uccAcuuccuAccuGAGGAGCATT B	2205
UUUGAGCAUGGAAGGAGGAUUCUG	2002	30786 KDR:3854U21 siRNA stab04 sense	B uGAGGcAuGGAAAGAGGAuicTT B	2053
AUGGUUUCUUGGCCUAGAAGGCCU	2018	30787 KDR:4089U21 siRNA stab04 sense	B GGiuucuGcucuAGAAAGAGTT B	2206
UCUGAAGGGCUCAAAACCAAGACAAG	2019	30788 KDR:4191U21 siRNA stab04 sense	B uGAAGGGucaAAAccAGAcATT B	2207
UGUCCACUUAUCCUGAGGAGCAAG	2017	30789 KDR:3094U21 siRNA (3076C) stab05 antisense	uGcuccuGGGuAAGUGGATsT	2208
UUUGAGCAUGGAAGGAGGAUUCUG	2002	30790 KDR:3872U21 siRNA (3894C) stab05 antisense	GAAuccucuuccuGcucATsT	2057
AUGGUUUCUUGGCCUAGAAGAGCU	2018	30791 KDR:4107U21 siRNA (4089C) stab05 antisense	cucuuccuGAGGCAAGAACccTsT	2209
UCUGAAGGGCUCAAAACCAAGACAAG	2019	30792 KDR:4209U21 siRNA (4191C) stab05 antisense	uGuuGGGuuGAGccuucATsT	2210
UGUCCACUUAUCCUGAGGAGCAAG	2017	31426 KDR:3076U21 siRNA sense	UCCACUUACCUGAGGAGCATT	2211
UUUGAGCAUGGAAGGAGGAUUCUG	2002	31435 KDR:3854U21 siRNA sense	UGAGCAUUGGAAGAGGAuUCTT	2045
AUGGUUUCUUGGCCUAGAAGGCCU	2018	31428 KDR:4089U21 siRNA sense	GGUUCUUGCCUCAGAAAGAGTT	2212
UCUGAAGGGCUCAAAACCAAGACAAG	2019	31429 KDR:4191U21 siRNA sense	UGAAGGGCUCAAAACCAGACATT	2213
UGUCCACUUAUCCUGAGGAGCAAG	2017	31430 KDR:3094U21 siRNA (3076C) antisense	UGCUCUCCAGGUAGUGGATT	2214
UUUGAGCAUGGAAGGAGGAUUCUG	2002	31439 KDR:3872U21 siRNA (3894C) antisense	GAAUCCUCUCCAGUCUATT	2049
AUGGUUUCUUGGCCUAGAAGAGCU	2018	31432 KDR:4107U21 siRNA (4089C) antisense	CUCUUCUAGGGCAAGAACCTT	2215
UCUGAAGGGCUCAAAACCAAGACAAG	2019	31433 KDR:4209U21 siRNA (4191C) antisense	UGUCUGGUUUGAGCCUUCATT	2216
UGACCUUUGGAGCAUCUCAUCUGU	2001	31434 KDR:3304U21 siRNA sense	ACCUUGGAGCAUCUCAUCUTT	2044
UCACCUUUCUUCUGUAUGGGAGGA	2003	31436 KDR:3894U21 siRNA sense	ACCUUUUUCUGUAUGGGATT	2046
GACAACACAGCAGGAAUCAGUCA	2004	31437 KDR:3948U21 siRNA sense	CAACACAGCAGGAAUCAGUTT	2047
UGACCUUUGGAGCAUCUCAUCUGU	2001	31438 KDR:3322U21 siRNA (3304C) antisense	AGAUGAGAUGCUCCAAGGGUTT	2048
UCACCUUUCUUCUGUAUGGGAGGA	2003	31440 KDR:3912U21 siRNA (3894C) antisense	CUCCAUACAGGAAACAGGGUTT	2050
GACAACACAGCAGGAAUCAGUCA	2004	31441 KDR:3966U21 siRNA (3948C)	ACUGAUUCCUGCUGGUUGTT	2051

			antisense		
GACAACACAGCAGGAAUCAGUCA	2004	31856	KDR:3948U21 siRNA stab04 sense	B cAACAcAGGAAucAGuTT B	2055
			KDR:3966U21 siRNA (3948C) stab05 antisense	AcUGAUuccuGcuGuGuuGtst	2059
GACAACACAGCAGGAAUCAGUCA	2004	31857	KDR:3854U21 siRNA stab07 sense	B uGAGCAuGGAGGGAAuucTT B	2061
UUUGAGCAUGGAAAGGAUUCUG	2002	31858	KDR:3948U21 siRNA stab07 sense	B cAACAcAGGAAucAGuTT B	2063
GACAACACAGCAGGAAUCAGUCA	2004	31859	KDR:3872U21 siRNA (3854C) stab08 antisense	GAAuccucuuucauGcucATsT	2226
UUUGAGCAUGGAAAGGAUUCUG	2002	31860	KDR:3966U21 siRNA (3948C) stab08 antisense	AcUGAUuccuGcuGuGuuGtst	2227
GACAACACAGCAGGAAUCAGUCA	2004	31861	KDR:3854U21 siRNA stab09 sense	B UGAGCAUGGAAAGGGAAuUcTT B	2228
UUUGAGCAUGGAAAGGAUUCUG	2002	31862	KDR:3948U21 siRNA stab09 sense	B CAAcACAGCAGGAAuCAGuTT B	2229
GACAACACAGCAGGAAUCAGUCA	2004	31863	KDR:3872U21 siRNA (3854C) stab10 antisense	GAAUCCUCUCCAUuGCUCATsT	2230
UUUGAGCAUGGAAAGGAUUCUG	2002	31864	KDR:3966U21 siRNA (3948C) stab10 antisense	ACUGAUUCCUGCUGuGUuGtst	2231
GACAACACAGCAGGAAUCAGUCA	2004	31865	KDR:3854U21 siRNA inv stab04 sense	B cuuAGGAGAAAGGUuAcGAGuTT B	2232
UUUGAGCAUGGAAAGGAUUCUG	2002	31878	KDR:3948U21 siRNA inv stab04 sense	B uGACuAAGGAcGAcAAcTT B	2233
GACAACACAGCAGGAAUCAGUCA	2004	31879	KDR:3872U21 siRNA (3854C) inv stab05 antisense	AcuGuAccuucuuccuAAAGTsT	2234
UUUGAGCAUGGAAAGGAUUCUG	2002	31880	KDR:3966U21 siRNA (3948C) inv stab05 antisense	GuuGuGuGuccuuAGucaTsT	2235
GACAACACAGCAGGAAUCAGUCA	2004	31881	KDR:3854U21 siRNA inv stab07 sense	B cuuAGGAGAAAGGUuAcGAGuTT B	2236
UUUGAGCAUGGAAAGGAUUCUG	2002	31882	KDR:3948U21 siRNA inv stab07 sense	B uGACuAAGGAcGAcAAcTT B	2237
GACAACACAGCAGGAAUCAGUCA	2004	31883	KDR:3872U21 siRNA (3854C) inv stab08 antisense	AcuGuAccuucuuccuAAAGTsT	2238
UUUGAGCAUGGAAAGGAUUCUG	2002	31884	KDR:3966U21 siRNA (3948C) inv stab08 antisense	GuuGuGuGuccuuAGucaTsT	2239
GACAACACAGCAGGAAUCAGUCA	2004	31885	KDR:3854U21 siRNA inv stab09 sense	B CUUAGGGAGGGuACGAGCAACTT B	2240
UUUGAGCAUGGAAAGGAUUCUG	2002	31886	KDR:3948U21 siRNA inv stab09 sense	ACUCGUACCUUCUCCuAAAGTsT	2242
GACAACACAGCAGGAAUCAGUCA	2004	31887	KDR:3872U21 siRNA (3854C) inv stab10 antisense	GUUGUGUCGUCCUuAGuCATsT	2243
CCUUUAUGGAUGCCAGCAAAU	2256	32238	KDR:2764U21 siRNA sense	CCUUUAUGGAUGCCAGCAAAUtt	2365
CUUAUGGAUGCCAGCAAAU	2257	32239	KDR:2765U21 siRNA sense	CUUAUGGAUGCCAGCAAAUgTT	2366
UUAUGGAUGCCAGCAAAUgg	2258	32240	KDR:2766U21 siRNA sense	UUAUGGAUGCCAGCAAAUgTT	2367

UAUGAUGCAGCAAUAGGG	2259	32241	KDR:2767U21 siRNA sense	UAUGAUGCCAGCAAUAGGGTT	2368
AUGAUGCCAGCAAUAGGA	2260	32242	KDR:2768U21 siRNA sense	AUGAUGCCAGCAAUAGGATT	2369
CAGACCAUGCUGGACUGCU	2261	32243	KDR:3712U21 siRNA sense	CAGACCAUGCUGGACUGCU	2370
AGACCAUGCUGGACUGCU	2262	32244	KDR:3713U21 siRNA sense	AGACCAUGCUGGACUGCU	2371
GACCAUGCUGGACUGCU	2263	32245	KDR:3714U21 siRNA sense	GACCAUGCUGGACUGCU	2372
ACCAUGCUGGACUGCU	2264	32246	KDR:3715U21 siRNA sense	ACCAUGCUGGACUGCU	2373
CCAUGCUGGACUGCU	2265	32247	KDR:3716U21 siRNA sense	CCAUGCUGGACUGCU	2374
CAGGAUGGCAAAGACUACAU	2266	32248	KDR:3811U21 siRNA sense	CAGGAUGGCAAAGACUACATT	2375
AGGAUGGCAAAGACUACAU	2267	32249	KDR:3812U21 siRNA sense	AGGAUGGCAAAGACUACATT	2376
CCUUAUGAUGCCAGCAAU	2256	32253	antisense	AUUGCUGGCAUCAUAGGTT	2377
CUUAUGAUGCCAGCAAU	2257	32254	antisense	CAUUGCUGGCAUCAUAGTT	2378
UUAUGAUGCCAGCAAU	2258	32255	antisense	CCAUUGCUGGCAUCAUATT	2379
UAUGAUGCCAGCAAU	2259	32256	antisense	CCCAUUUGCUGGCAUCAUATT	2380
AUGAUGCCAGCAAU	2260	32257	antisense	UCCCCAUUUUGCUGGCAUCAU	2381
CAGACCAUGCUGGACUGCU	2261	32258	antisense	AGCAGUCCAGCAUGGUCUGTT	2382
AGACCAUGCUGGACUGCU	2262	32259	antisense	CAGCAGUCCAGCAUGGUCUTT	2383
GACCAUGCUGGACUGCU	2263	32260	antisense	CCAGCAGUCCAGCAUGGUCUTT	2384
ACCAUGCUGGACUGCU	2264	32261	antisense	GCCAGCAGUCCAGCAUGGUCUTT	2385
CCAUGCUGGACUGCU	2265	32262	antisense	UGCCAGCAGUCCAGCAUGGUCUTT	2386
CAGGAUGGCAAAGACUACAU	2266	32263	antisense	UGUAGCUUUJGCCAUCUGTT	2387
AGGAUGGCAAAGACUACAU	2267	32264	antisense	AUGUAGCUUUJGCCAUCUGTT	2388
UGACCUUGGAGCAUCUCAUCUGU	2001	32310	KDR:3304U21 siRNA stab09 sense	B ACCUUGGAGCAUCUCAUCUTT B	2389
UCACCUUUUCCUGUAUGGAGGA	2003	32311	KDR:3894U21 siRNA stab09 sense	B ACCUUGUUUCCUGUAUGGAGTT B	2390
UGACCUUGGAGCAUCUCAUCUGU	2001	32312	antisense	KDR:3322U21 siRNA (3304C) stab10	2391
UCACCUUUUCCUGUAUGGAGGA	2003	32313	antisense	AGAUGAGAUGCUCCAAAGGUTsT	2392
UGACCUUGGAGCAUCUCAUCUGU	2001	32314	sense	CUCCAUACAGGAAACAGGUTsT	2393
UCACCUUUUCCUGUAUGGAGGA	2003	32315	sense	B UCUACUCUACGAGGUUCCATT B	2394

UGACCUUGGAGCAUCUCAUCUGU	2001	32316	KDR:3322L21 siRNA (3304C) inv stab10 antisense KDR:3912L21 siRNA (3894C) inv stab10 antisense	UGGAACCUUGGUAGAGUAGATsT	2395
UCACCUGUUUCUGUAGGGAGGA	2003	32317	KDR:828U21 siRNA stab07 sense KDR:3912L21 siRNA stab07 sense	UGGACAAAGGACAUACCUCUTsT	2396
AACAGAAUUUUCCUGGGACACCAA	2268	32762	KDR:3758U21 siRNA stab07 sense KDR:3912L21 siRNA stab07 sense	BcAGAAuuiucGGGAcAGCtT B	2397
UGGAGCAUCUCAUCGUUACAGC	2269	32763	KDR:3310U21 siRNA stab07 sense KDR:3758U21 siRNA stab07 sense	BGAGcAucuCAucuGuuACATT B	2398
CACGUUUUCAGUUGGGAGAC	2270	32764	KDR:3758U21 siRNA stab07 sense KDR:3912L21 siRNA stab07 sense	BcGuuucAGAGuGGuGGATT B	2399
CUCACCUGUUUCUGUUAUGGAGG	2271	32765	KDR:3893U21 siRNA stab07 sense KDR:846L21 siRNA (828C) stab08 antisense	BcAccuGuuucGuuAGGGATT B	2400
AACAGAAUUUUCCUGGGACACCAA	2268	32767	KDR:3328L21 siRNA (3310C) stab08 antisense	GcuGuuccAGGAAuucuGTsT	2401
UGGAGCAUCUCAUCGUUACAGC	2269	32768	KDR:3776L21 siRNA (3758C) stab08 antisense	uGuuACAGAGuGAGAUGuGcuctsT	2402
CACGUUUUCAGUUGGGAGAC	2270	32769	KDR:3911L21 siRNA (3893C) stab08 antisense	uccAccAAcucuGAAAACGTsT	2403
CUCACCUGUUUCUGUUAUGGAGG	2271	32770	KDR:3912L21 siRNA (3894C) stab08 antisense	uccAUACAGGAAACAGGUGTsT	2404
UCACCUGUUUCUGUUAUGGAGG	2003	32771	KDR:828U21 siRNA inv stab07 sense KDR:3910U21 siRNA inv stab07 sense	cuccAUAcAGGAAACAGGUtsT	2405
AACAGAAUUUUCCUGGGACACCAA	2268	32786	KDR:3310U21 siRNA inv stab07 sense	BcGACAGGGGuuuaAGAcTT B	2406
UGGAGCAUCUCAUCGUUACAGC	2269	32787	KDR:3758U21 siRNA inv stab07 sense	BAcAuuGuuAcucuAcGAGTT B	2407
CACGUUUUCAGUUGGGAGAC	2270	32788	KDR:3893U21 siRNA inv stab07 sense	BAGGUGuuGAGAcuuuGuGCTT B	2408
CUCACCUGUUUCUGUUAUGGAGG	2271	32789	KDR:3894U21 siRNA inv stab07 sense	BAGGGGuuGuuccuuGuccATT B	2409
UCACCUGUUUCUGUUAUGGAGG	2003	32790	KDR:846L21 siRNA (828C) inv stab08 antisense	B GAGGUuGuuccuuGuccATT B	2410
AACAGAAUUUUCCUGGGACACCAA	2268	32791	KDR:3328L21 siRNA (3310C) inv stab08 antisense	GuuuaAAAGGAaccuGuGcGTsT	2411
UGGAGCAUCUCAUCGUUACAGC	2269	32792	KDR:3776L21 siRNA (3758C) inv stab08 antisense	cucGuAGAGuAGAcAAuGuJstT	2412
CACGUUUUCAGUUGGGAGAC	2270	32793	KDR:3911L21 siRNA (3893C) inv stab08 antisense	GcAAAGGuuAAAGGAacAccuTsT	2413
CUCACCUGUUUCUGUUAUGGAGG	2271	32794	KDR:3912L21 siRNA (3894C) inv stab08 antisense	GuGGGacAAAGGAacAuAccuTsT	2414
UCACCUGUUUCUGUUAUGGAGG	2003	32795	KDR:3912L21 siRNA (3894C) inv stab08 antisense	uGGGacAAAGGAacAuAccuTsT	2415
AACAGAAUUUUCCUGGGACACCAA	2268	32958	KDR:828U21 siRNA stab09 sense KDR:3310U21 siRNA stab09 sense	B CAGAAUUUCUGGGACAGCTT B	2416
UGGAGCAUCUCAUCGUUACAGC	2269	32959	KDR:3758U21 siRNA stab09 sense	B GAGCAUCUCAUCGUuACATT B	2417
CACGUUUUCAGUUGGGAGAC	2270	32960	KDR:3758U21 siRNA stab09 sense	B CGUUUUCAGGUUGGGATT B	2418
CUCACCUGUUUCUGUUAUGGAGG	2271	32961	KDR:3893U21 siRNA stab09 sense KDR:846L21 siRNA (828C) stab10 antisense	B CACCUGUUUCUGUUAUGGATT B	2419
AACAGAAUUUUCCUGGGACACCAA	2268	32963		GCUGUCCCCAGGAAAUUCUGTsT	2420

UGGAGCAUCUCAUCUUACAGC	2269	32964	KDR:3328L21 siRNA (3310C) stab10 antisense	UGUAACAGAUGAGAUGCUCTsT	2421
CACGUUUUCAGAGUUGGGAAC	2270	32965	KDR:3776L21 siRNA (3758C) stab10 antisense	UCACCAACUCUGAAAACGTsT	2422
CUCACCUGUUUCUGUAGGAGG	2271	32966	KDR:3911L21 siRNA (3893C) stab10 antisense	UCCAUACAGGAAACAGGUGTsT	2423
AACAGAAUUUCCUGGGACGCAA	2268	32988	KDR:828U21 siRNA inv stab09 sense	B CGACGGGUUUUAAGACTT B	2424
UGGAGCAUCUCAUCUUACAGC	2269	32989	KDR:3310U21 siRNA inv stab09 sense	B ACAUUGUCUACUCUACGAGTT B	2425
CACGUUUUCAGAGUUGGGAAC	2270	32990	KDR:3758U21 siRNA inv stab09 sense	B AGGGGGUUGAGACUUUJUGCTT B	2426
CUCACCUGUUUCUGUAGGAGG	2271	32991	KDR:3893U21 siRNA inv stab09 sense	B AGGU AUGGUCCUUUGUCCACTT B	2427
AACAGAAUUUCCUGGGACGCAA	2268	32993	KDR:846L21 siRNA (828C) inv stab10 antisense	GUUUAAAAGGACCCUGUCGTsT	2428
UGGAGCAUCUCAUCUUACAGC	2269	32994	KDR:3328L21 siRNA (3310C) inv stab10 antisense	CUCGUAGAGUAGACAUGUTsT	2429
CACGUUUUCAGAGUUGGGAAC	2270	32995	KDR:3776L21 siRNA (3758C) inv stab10 antisense	GCAAAAGUCUCAACCACCTsT	2430
CUCACCUGUUUCUGUAGGAGG	2271	32996	KDR:3911L21 siRNA (3893C) inv stab10 antisense	GUUGACAAAGGACAUACCUUTsT	2431

VEGFR3

Target	Seq ID	COMPOUND#	Aliases	Sequence	Seq ID
AGCACUGCCACAAGGAAGUACUG	2005	31904	FLT4:2011U21 siRNA sense	CACUGCCACAAGGAAGUACCTT	2068
CUGAAGCAGAGAGAGAGAAAGCA	2006		FLT4:3921U21 siRNA sense	GAAGCAGAGAGAGAGAAAGGTT	2069
AAAGAGGAACCGAGGAGACAAGA	2007		FLT4:4038U21 siRNA sense	AGAGGAACCGAGGAGGACAAATT	2070
GACAAGGGAGCAUGAAAAGUGGA	2008		FLT4:4054U21 siRNA sense	CAAGAGGGAGCAUGAAAAGUGTT	2071
AGCACUGCCACAAGGAAGUACUG	2005	31908	antisense	GGUACUUCUUGUGGCAGUGTT	2072
CUGAAGCAGAGAGAGAGAAAGGCA	2006		FLT4:3939U21 siRNA (3921C) antisense	CCUUCUCUCUCUCUGCUUCUTT	2073
AAAGAGGAACCGAGGAGACAAGA	2007		FLT4:4056U21 siRNA (4038C) antisense	UUGGUCCUCGGUUCUCUTT	2074
GACAAGGGAGCAUGAAAAGUGGA	2008		FLT4:4072U21 siRNA (4054C) antisense	CACUUUCAUGCUCCUCUUGTT	2075
AGCACUGCCACAAGGAAGUACUG	2005		FLT4:2011U21 siRNA stab04 sense	B CACuGccAcAAGAGuAcCTT B	2076
CUGAAGCAGAGAGAGAGAAAGGCA	2006		FLT4:3921U21 siRNA stab04 sense	B GAAAGCAGAGAGAGAGAAAGGTT	
AAAGAGGAACCGAGGAGACAAGA	2007		FLT4:4038U21 siRNA stab04 sense	B AGAGGAACCGAGGAGAAATT B	2078
GACAAGGGAGGAGCAUGAAAAGUGGA	2008		FLT4:4054U21 siRNA stab04 sense	B cAAGAGGGAGcaUGAAAAGUGTT B	2079
AGCACUGCCACAAGGAAGUACUG	2005		FLT4:3929U21 siRNA (2011C) stab05 antisense	GGuAcuUuUGGGAGUGTst	2080
CUGAAGCAGAGAGAGAAAGGCA	2006		FLT4:4056U21 siRNA (4038C) stab05 antisense	ccuucucucucucuGcuuicTsT	2081
AAAGAGGAACCGAGGAGACAAGA	2007		FLT4:4056U21 siRNA (4054C) stab05 antisense	uuGuccuccuGGuuccuicTsT	2082
GACAAGGGAGCAUGAAAAGUGGA	2008		FLT4:3939U21 siRNA (3921C) stab05 antisense	cAuuuuAuGuccuccuicTsT	2083
AGCACUGCCACAAGGAAGUACUG	2005		FLT4:2011U21 siRNA stab07 sense	B CACuGccAcAAGAGuAcCTT B	2084
CUGAAGCAGAGAGAGAGAAAGGCA	2006		FLT4:4072U21 siRNA (4054C) stab05 antisense	B GAAGCAGAGAGAGAAAGGTT	
AAAGAGGAACCGAGGAGACAAGA	2007		FLT4:4038U21 siRNA stab07 sense	B AGAGGAACCGAGGAGAAATT B	2085
GACAAGGGAGGAGCAUGAAAAGUGGA	2008		FLT4:4054U21 siRNA stab07 sense	B cAAGAGGGAGcaUGAAAAGUGTT B	2087
AGCACUGCCACAAGGAAGUACUG	2005		FLT4:3921U21 siRNA (2011C) stab11 antisense	GGuAcuUuUGGGAGUGTst	2088
CUGAAGCAGAGAGAGAAAGGCA	2006		FLT4:3939U21 siRNA (3921C) stab11 antisense	ccuucucucucuGcuuicTsT	2089
AAAGAGGAACCGAGGAGACAAGA	2007		FLT4:4056U21 siRNA (4038C) stab11 antisense	uuGuccuccuGGGuuccuicTsT	2090
GACAAGGGAGCAUGAAAAGUGGA	2008		FLT4:4072U21 siRNA (4054C) stab11 antisense	cAuuuuAuGuccuccuicTsT	2091

ACUUUUAUGUGACCAUCUCCC	2272	31902	FLT4:1666U21 siRNA sense	UUCUAUGUGACCAUCUCC	2432
CAAGCACUGCCACAAGGUACC	2273	31903	FLT4:2009U21 siRNA sense	AGCACUGGCCACAAGGUATT	2433
AGUACGGCAACCUCCUCUCAACUUC	2274	31905	FLT4:2815U21 siRNA sense	UACGGCAACCUCCUCAACUTT	2434
ACUUUUAUGUGACCAUCUCCC	2272	31906	FLT4:1684L21 siRNA (1666C) antisense	GGAUUGGGGGUACAUAGAAATT	2435
CAAGCACUGCCACAAGGUACC	2273	31907	FLT4:2027L21 siRNA (2009C) antisense	UACUUUCUUGGGCAGUGCUTT	2436
AGUACGGCAACCUCCUCAACUUC	2274	31909	FLT4:2833L21 siRNA (2815C) antisense	AGUUGGAGGGGUUGCCGUATT	2437

Uppercase = ribonucleotide

u,c = 2'-deoxy-2'-fluoro U,C

T = thymidine

B = inverted deoxy abasic

S = phosphorothioate linkage

A = deoxy Adenosine

G = deoxy Guanosine

A = 2'-O-methyl Adenosine

G = 2'-O-methyl Guanosine

X= nitroindole universal base

Z= nitropyrole universal base

Y= 3',3'-inverted thymidine

M= glyceryl

N= 3'-O-methyl uridine

P= L-thymidine

Q= L-uridine

R= 5-bromo-deoxy-uridine

Z = sbL: symmetrical
bifunctional linkerH = chol2: capped Cholesterol
TEG

Table IV

Non-limiting examples of Stabilization Chemistries for chemically modified siRNA constructs

Chemistry	pyrimidine	Purine	cap	p=S	Strand
“Stab 1”	Ribo	Ribo	-	5 at 5'-end 1 at 3'-end	S/AS
“Stab 2”	Ribo	Ribo	-	All linkages	Usually AS
“Stab 3”	2'-fluoro	Ribo	-	4 at 5'-end 4 at 3'-end	Usually S
“Stab 4”	2'-fluoro	Ribo	5' and 3'-ends	-	Usually S
“Stab 5”	2'-fluoro	Ribo	-	1 at 3'-end	Usually AS
“Stab 6”	2'-O-Methyl	Ribo	5' and 3'-ends	-	Usually S
“Stab 7”	2'-fluoro	2'-deoxy	5' and 3'-ends	-	Usually S
“Stab 8”	2'-fluoro	2'-O-Methyl	-	1 at 3'-end	Usually AS
“Stab 9”	Ribo	Ribo	5' and 3'-ends	-	Usually S
“Stab 10”	Ribo	Ribo	-	1 at 3'-end	Usually AS
“Stab 11”	2'-fluoro	2'-deoxy	-	1 at 3'-end	Usually AS
“Stab 12”	2'-fluoro	LNA	5' and 3'-ends	-	Usually S
“Stab 13”	2'-fluoro	LNA	-	1 at 3'-end	Usually AS
“Stab 14”	2'-fluoro	2'-deoxy	-	2 at 5'-end 1 at 3'-end	Usually AS
“Stab 15”	2'-deoxy	2'-deoxy	-	2 at 5'-end 1 at 3'-end	Usually AS
“Stab 16”	Ribo	2'-O-Methyl	5' and 3'-ends	-	Usually S
“Stab 17”	2'-O-Methyl	2'-O-Methyl	5' and 3'-	-	Usually S

			ends	
“Stab 18”	2'-fluoro	2'-O-Methyl	5' and 3'-ends	1 at 3'-end
“Stab 19”	Ribo	Ribo	TT at 3'-ends	S/AS
“Stab 20”	Ribo	Ribo	TT at 3'-ends	1 at 3'-end

CAP = any terminal cap, see for example **Figure 10**.

All Stab 1-20 chemistries can comprise 3'-terminal thymidine (TT) residues

All Stab 1-20 chemistries typically comprise 21 nucleotides, but can vary as described herein.

S = sense strand

AS = antisense strand

Table VA. 2.5 μ mol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 μ L	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 μ L	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 μ L	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 μ L	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 μ L	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2 μ mol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 μ L	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 μ L	45 sec	233 min	465 sec
Acetic Anhydride	655	124 μ L	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 μ L	5 sec	5 sec	5 sec
TCA	700	732 μ L	10 sec	10 sec	10 sec
Iodine	20.6	244 μ L	15 sec	15 sec	15 sec
Beaucage	7.7	232 μ L	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 μ mol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O- methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O- methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 μ L	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 μ L	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 μ L	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 μ L	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 μ L	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 μ L	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 μ L	NA	NA	NA

5 • Wait time does not include contact time during delivery.

• Tandem synthesis utilizes double coupling of linker molecule